Dalila do Rosário Encarnação Serpa

Licenciada em Biologia Aplicada aos Recursos Animais – Variante Recursos Marinhos

Macroalgal (*Enteromorpha* spp. and *Ulva* spp.) Primary Productivity in the Ria Formosa Lagoon

Dissertação para obtenção do grau de Mestre em
Ecologia, Gestão e Modelação dos Recursos Marinhos

Orientador: Professor Doutor João Pedro Salgueiro Gomes Ferreira,
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Júri:

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Resumo

Em áreas sujeitas a eutrofização, as macroalgas oportunistas de crescimento rápido e elevadas taxas de consumo de nutrientes, podem tornar-se os principais produtores primários do ecossistema, pelo que se torna de grande importância a avaliação correcta da produtividade anual destas algas. Experiências de incubação *in situ* foram realizadas de modo a determinar quais os factores ambientais que controlam a produtividade das espécies de *Enteromorpha* e *Ulva* na laguna da Ria Formosa. A análise de regressão revelou que o padrão de variação sazonal da produtividade das algas está significativamente ($p < 0.05$) relacionado com a intensidade luminosa, sendo que as taxas fotossintéticas mais elevadas são observadas durante o período de outono/principio de Inverno. Em laboratório, foram também realizadas experiências de incubação de modo a determinar os parâmetros das curvas *P-I* para estas algas. Em ambas as experiências, *in situ* e em laboratório, observou-se fotoinibição a intensidades luminosas mais elevadas. A taxa máxima de produção para a *Ulva* foi de 3.66 mg C g^{-1} dw h^{-1}, enquanto que para a *Enteromorpha* o valor deste parâmetro foi de 3.55 mg C g^{-1} dw h^{-1}. Os valores da intensidade luminosa óptima para a fotossíntese eram semelhantes para a *Enteromorpha* (47 µE m^{-2} s^{-1}) e para a *Ulva* (35 µE m^{-2} s^{-1}). A relação entre a luz e as taxas de consumo de nutrientes das macroalgas foi também estudada em laboratório, contudo esta relação não foi significativa ($p > 0.05$), provavelmente devido ao facto destas algas oportunistas apresentarem "consumo de luxo". As taxas respiratórias da *Enteromorpha* variaram entre 0.04 e 0.25 mg C g^{-1} dw h^{-1}, enquanto que para a *Ulva* os valores foram ligeiramente superiores, variando entre 0.08 e 0.35 mg C g^{-1} dw h^{-1}. Para simular a produtividade anual das macroalgas dominantes na laguna, desenvolveu-se um modelo ecológico. O modelo incluiu a inibição pela luz e a limitação de nutrientes tendo em conta as concentrações internas de azoto e fósforo. Para ambas as algas observou-se que o azoto não era um nutriente limitativo para a fotossíntese, pois ao longo do ano as concentrações internas deste nutriente foram sempre superiores à quota interna mínima. Contudo, observou-se que as concentrações internas de fósforo diminuíam durante o período de Inverno provavelmente devido à baixa disponibilidade de fósforo dissolvido na água. Os valores de produção primária bruta anual estimados pelo modelo são de 190 g C m^{-2} ano^{-1} para a *Enteromorpha* e de 132 g C m^{-2} ano^{-1} para a *Ulva*. Anualmente estas algas contribuem com cerca de 446 ton de carbono para o ecossistema, dos quais 85% provêem das espécies de *Enteromorpha*. As macroalgas bentónicas são também importantes na remoção de nutrientes, removendo aproximadamente 69 toneladas de azoto e 10 toneladas de fósforo, anualmente. Estas quantidades correspondem, em termos de habitantes equivalentes, a cerca de 15700 habitantes para o azoto e 10000 habitantes para o fósforo.

Palavras-Chave: Macroalgas; Curvas P-I; Produtividade primária; Consumo de nutrientes; Modelação ecológica

Abstract

In areas undergoing eutrophication, opportunistic macroalgae with high nutrient uptake rates and rapid growth may become the dominant primary producers of the ecosystem, revealing the importance of an accurate evaluation for the annual algal production. In order to determine the environmental factors controlling *Enteromorpha* and *Ulva* species productivity in the Ria Formosa lagoon, *in situ* short-term incubation experiments were performed. Regression analysis revealed that light seems to accurately ($p<0.05$) explain the pattern of seasonal variation of algae productivity, with highest photosynthetic rates during the autumn/early winter period. Laboratory incubation experiments were performed to determine *P-I* curves parameters for both species. In both *in situ* and laboratory experiments, seaweed exhibited photoinhibition at high light intensities. Maximum production rate for *Ulva* was 3.66 mg C g^{-1} dw h^{-1}, while for *Enteromorpha* it was 3.55 mg C g^{-1} dw h^{-1}. Similar values of optimal light intensity were estimated for *Enteromorpha* (47 µE m^{-2} s^{-1}) and *Ulva* (35 µE m^{-2} s^{-1}) species. The relationship between macroalgal nutrient uptake rates and light irradiances were also evaluated in the laboratory experiments, but the results of regression analysis showed that it was not significant ($p > 0.05$), probably due to "luxury consumption" by these opportunistic macroalgae. *Enteromorpha* respiratory rates ranged from 0.04 to 0.25 mg C g^{-1} dw h^{-1}, while for *Ulva* respiration values were slightly higher varying between 0.08 and 0.35 mg C g^{-1} dw h^{-1}. An ecological model was developed in order to simulate the annual productivity of the dominant macroalgae species in the lagoon. The model included photoinhibition and nutrient limitation based on the internal concentration of nitrogen and phosphorus. For both seaweeds there was no nitrogen limitation for seaweed productivity. Over the year, internal nitrogen concentrations were always higher than the minimum internal quota. However, internal phosphorus concentrations were depleted during the winter period probably due to the low availability of dissolved phosphorus. Values of annual gross primary production estimated by the model are higher for *Enteromorpha* spp. (190 g C m^{-2} y^{-1}) than for *Ulva* spp. (132 g C m^{-2} y^{-1}). The seaweeds contribution to the lagoon carbon budget is of 446 ton C y^{-1}, from which 85 % correspond to *Enteromorpha* species. Benthic macroalgae also play an important role in nutrient removal. Annually, these algae remove about 69 tons of nitrogen and 10 tons of phosphorus. These quantities correspond in terms of population equivalents to 15700 inhabitants for nitrogen and 10000 inhabitants for phosphorus.

Keywords: Macroalgae; P-I curves; Primary productivity; Nutrient uptake; Ecological modelling

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List of Symbols

RF – Ria Formosa

k – light extinction coefficient

SPM – suspended particulate matter

DAIN – dissolved available inorganic nitrogen

P – photosynthesis

I – light intensity

P_n – net primary productivity

P_g – gross primary productivity

R – respiration

P_{max} – maximum production rate

α - initial slope

I_{opt} – optimal light intensity

I_k – saturating irradiance

I_c – compensation light intensity

PAR – Photosynthetically Active Radiation

NH_4^+ – ammonium

NO_3^{-2} – nitrate

NO_2^- – nitrite

HPO_4^{-2} – phosphate

dw – dry weight

C.V. – variation coefficient

fw – fresh weight

B – biomass

M – mortality

I_0 – light energy available at water surface

I_z – light available for photosynthesis at depth z

z – depth

h – height of the macroalgae stand above datum

HT – high tide

LT – low tide

t – time

max HT – maximum water level at high tide

min HT – minimum water level at high tide

max LT – maximum water level at low tide

min LT – minimum water level at low tide

N – internal nitrogen concentration

P – internal phosphorus concentration

N_{up} – nitrogen uptake

N_{fb} – nitrogen feedback control

P_{up} – phosphorus uptake

P_{fb} – phosphorus feedback control

VN_{max} – maximum uptake rate for nitrogen

TIN – total inorganic nitrogen

K_N – half saturation constant for nitrogen

VP_{max} – maximum uptake rate for phosphorus

K_P – half saturation constant for phosphorus

QN_{max} – maximum internal quota for nitrogen

QN_{min} – minimum internal quota for nitrogen

QP_{max} – maximum internal quota for phosphorus

QP_{min} – minimum internal quota for phosphorus

Max_{mort} – maximum mortality rate

K_m – mortality rate

CHAPTER I.

General Introduction

1. General Introduction

In recent years, the quality of coastal waters worldwide has deteriorated as a result of an increase of human population and activities along coastal regions (Newton *et al.*, 2003). Anthropogenic activities, such as deforestation, agriculture, animal rearing and wastewater treatment, increase the nutrient supply to coastal ecosystems leading to eutrophication (Fong *et al.*, 1994).

Under eutrophic conditions, an excessive growth of seaweeds is observed (Morand and Briand, 1996) and these algae may become the dominant primary producers of the ecosystem (Peckol and Rivers, 1996; Kinney and Roman, 1998; Martins *et al.*, 2001), outcompeting other species, such as seagrasses and phytoplankton. The competition for nutrients can also control the algal community structure by favouring opportunistic macroalgae species, such as *Enteromorpha* and *Ulva*, with high nutrient uptake rates and rapid growth (Fong *et al.*, 1994; Pedersen and Borum, 1996; Bachelet *et al.*, 2000) due to a simple thallus morphology (high surface to weight ratios) which makes them more suited to obtain light energy and nutrients (Littler, 1979) than other benthic macroalgae.

At first, the development of opportunistic macroalgae acts as a bioremediation mechanism (Morand and Briand, 1996) because a large part of the nutrients excess is removed. Then, when the environmental conditions become unfavourable, the plants die and decompose, leading to an increase in the amounts of organic detritus and a decrease in dissolved oxygen concentrations.
This degradation of water quality affects the living resources, tourism, recreation, and human and environmental health.

Besides nutrient availability, the macroalgal primary productivity is also determined by other environmental factors such as light intensity and temperature (Parsons *et al.*, 1984; JØrgensen, 1994).

In shallow coastal ecosystems such as the Ria Formosa lagoon, where light may reach the bottom, benthic primary producers play an important role in carbon fixation and nutrient removal (NICE, 1999). Thus, the study of macroalgal primary productivity is of special interest once it can be used as a potential indicator of eutrophication (Fong *et al.*, 1994).

Recently, studies carried out in the Ria Formosa lagoon (Newton *et al.*, 2003) demonstrated the potential for episodic eutrophic conditions in the lagoon, as a response to anthropogenic nutrient loading, mainly from sewage contamination and agricultural run-off. According to other authors

(Padinha *et al.*, 2000) there are already signs of eutrophication, as it was documented a decrease in seagrasses and a large increase in algal mats in the lagoon. The macroalgal community of the Ria Formosa is also dominated by green opportunistic macroalgae, such as the Ulvales, *Enteromorpha* spp. and *Ulva* spp. (Cunha, 1990; Aníbal, 1998), species often found in areas undergoing eutrophication (Lavery and McComb, 1991; Sfriso and Marcomini, 1997; Martins *et al.*, 2001).

The benthic macroalgae community of the Ria Formosa lagoon is not well studied. Although there are some species inventories (Ardré, 1970; Cunha, 1990; Duarte *et al.*, 1988), studies on seaweeds taxonomy (Mata, 1997), and on the spatial and seasonal variability of algae biomass over the year (Reis, 1994; Aníbal, 1998), there is a lack of information about macroalgal productivity and their contribution to the overall ecosystem productivity.

2. Objectives

The main objective of the present work was to the estimate the annual productivity of the dominant macroalgae species (*Enteromorpha* spp. and *Ulva* spp.) in the Ria Formosa lagoon. This was approached by:

1) Determining the relative importance of environmental factors (water temperature, light intensity and salinity) controlling macroalgal productivity in the Ria Formosa;
2) Determining the *P-I* curve parameters for each macroalgae;
3) Developing an ecological model in order to simulate the annual macroalgal productivity at a small spatial scale.

CHAPTER II.

Site description

Site description

The Ria Formosa is a shallow mesotidal lagoon located along the eastern part of the south coast of Portugal (Fig. 1.1), with an extension of 55 km (from Ancão to Cacela), a maximum width of 6 km (from Faro to Cape Santa Maria) and a wet area of 105 km^2. The lagoon is protected from the ocean by a sandy barrier island interrupted by six inlets (S.Luís, Faro-Olhão, Armona, Fuzeta, Tavira e Cacela) (Fig. 1.1). It has several channels and an extensive intertidal area, around 50 % of the total wet area (53 km^2), mostly constituted by sand, muddy-sand flats and salt marshes (Bettencourt, 1994).

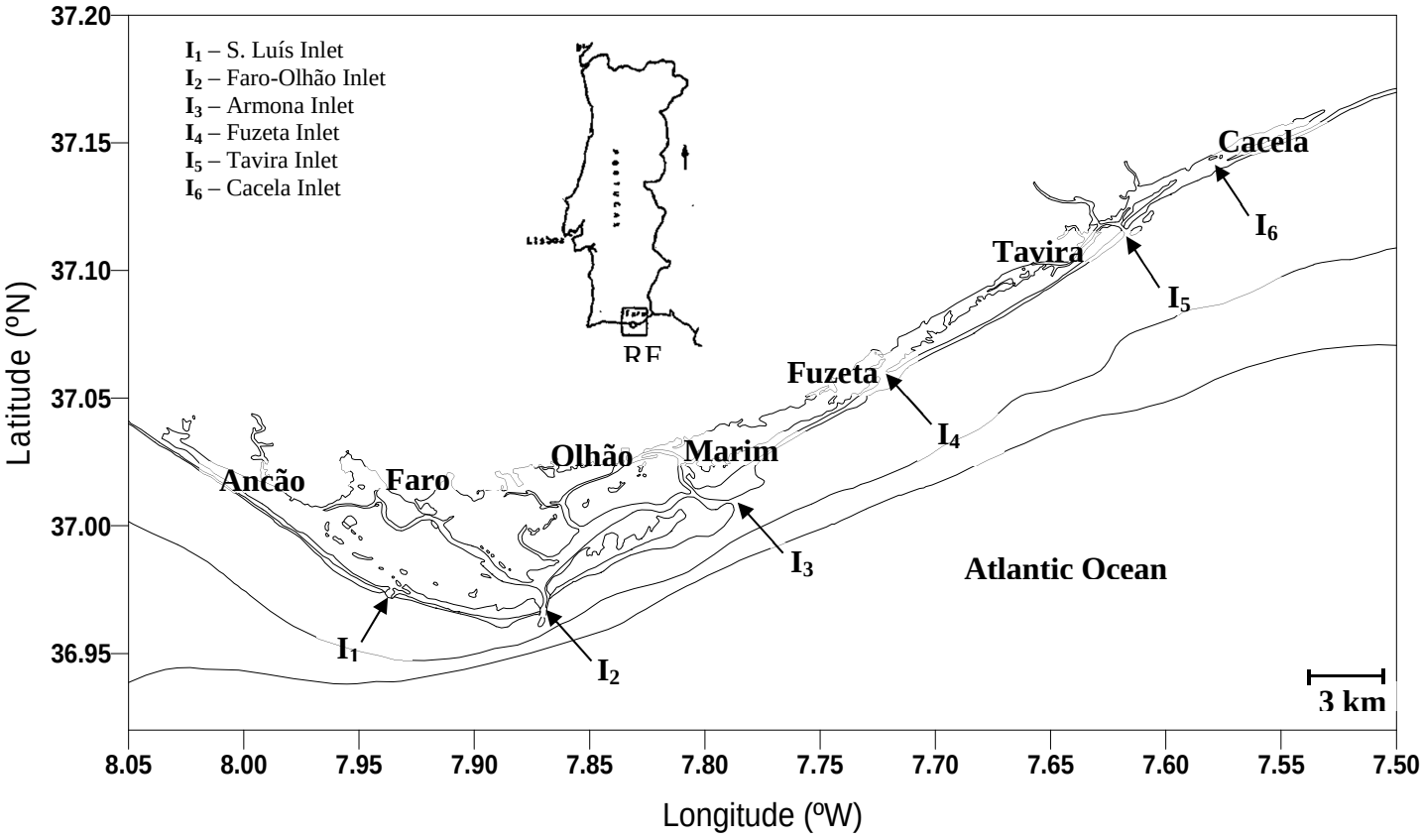

Figure 2.1 - Geographic location of the Ria Formosa lagoon.

The morphological diversity of this ecosystem determines different environmental units defined according to morphological and phytomorphological criteria (Andrade, 1990). These environmental units are classified as:

Tidal flats establish the connection between salt marshes and tidal channels. Their lower limit is the mean level of the low tides of spring tides. These areas are characterized by silt-clay or muddy-sand bottoms without halophyte vegetation but abundantly covered by the seagrass,

Zostera noltii. Almost 5 km^2 of the tidal flats are used for clam farming, which means removal of vegetation and a periodical addition of sand.

Salt marshes located above tidal flats cover an area of 34 km^2. Silt-clay sediments colonised by halophyte species characterize these areas. The vegetation is dominated by *Spartina maritima*, *Salicornia nitens*, *Arthrocnemum perenne*, *Suaeda maritima* and *Atriplex portucaloides* [1]. The low salt marsh is almost exclusively colonized by *Spartina maritima* and the bigger plants colonize the high salt marsh with a shorter immersion period.

Tidal channels allow an easy water circulation. The bottom grain size varies from coarse sand to silt. Large areas of the bottom are covered by *Zostera marina*, which contributes to sedimentation of suspended particulate matter.

Lagoon beaches with sand and muddy-sand sediments appear especially within tidal flats and salt marshes.

The Ria Formosa lagoon and some of its hinterland has been included in a National Park (184 km^2) and accepted as a Natura 2000 network and a Ramsar site, as a recognition of its environmental value. The park includes a great diversity of habitats, including salt marshes (35 km^2), dunes (19 km^2), saltpans (10 km^2), fish farms (2.8 km^2) and, muddy-sand flats (24 km^2) [1]. The lagoon is also a valuable resource to the Algarve region for tourism, fisheries, aquaculture and salt extraction.

1. Climate

The climate in the Algarve region is typically Mediterranean, with warm dry summers and mild winters.

1.1 Temperature

In the eastern part of the lagoon, the mean annual temperature ranges from 16 ºC to 17.5 ºC, while in the western part of the lagoon it ranges from 17.5 ºC to 20 ºC (Fig. 1.2). Minimum temperature values are observed in January and February (8 - 9 ºC) and maximum values in July-August (29 – 30 ºC) (Falcão, 1997).

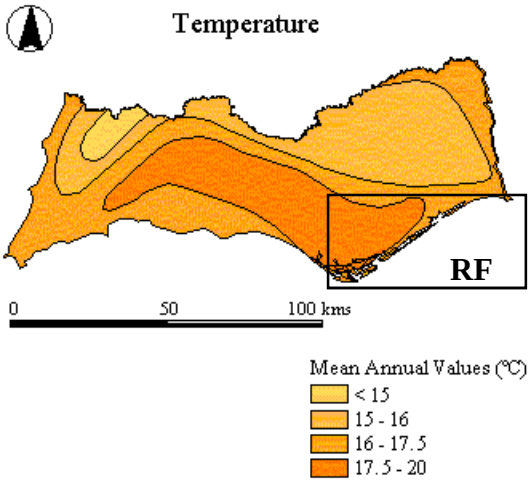

Figure 2.2 - Mean annual temperature (ºC) in the Ria Formosa lagoon [2].

1.2 Rainfall

In the Ria Formosa, the mean annual values of rainfall vary between 400 and 600 mm y^{-1} (Fig. 1.3). Generally, the wettest month is December with about 17% of the total annual rainfall, and the driest months are July and August with less than 1% of the annual rainfall (Falcão, 1997).

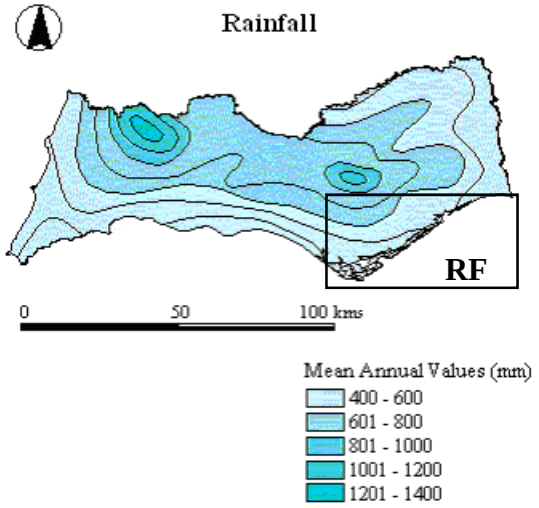

Figure 2.3 - Mean annual values of rainfall (mm y^{-1}) in the Ria Formosa lagoon [2].

1.3 Solar radiation and insolation

In the Ria Formosa, solar radiation is high with mean annual values ranging between 161 and 165 kcal cm^{-2}, except in the extreme west of the lagoon where values above 165 kcal cm^{-2} are reached (Fig. 1.4). Insolation is also high varying between 3000 and 3200 hours in a year [2].

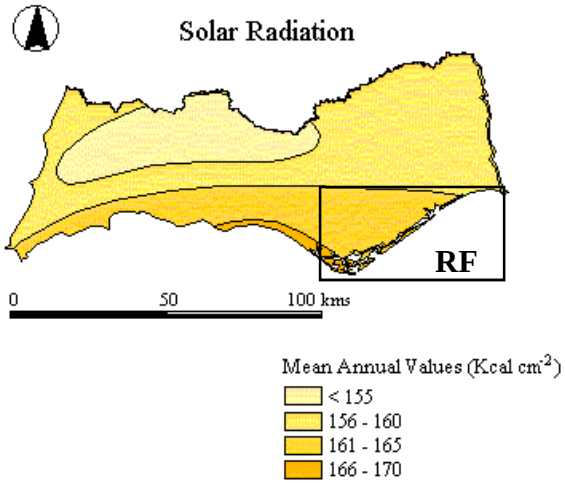

Figure 2.4 - Mean annual values of solar radiation (kcal cm^{-2}) in the Ria Formosa lagoon [2].

1.4 Wind

In the eastern part of the Algarve region where Ria Formosa is located, the wind blows predominantly from west and southwest, however during spring and autumn there is a high incidence of winds blowing from east (Granja, 1984).

2. Hydrodynamics

As the volume of water exchanged between the lagoon and the sea varies from 30×10^6 to 100×10^6 m^3, according to the tidal amplitude (CEPASA, 1980), and the subtidal water volume is about 50×10^6 m^3, it is assumed that at neap and spring tides, 40% to 70% of the water volume is respectively exchanged with the sea (Sprung, 1994).

2.1 Currents

In the main channels the current speed is less than 1 m s^{-1}, while in the inlets it exceeds 2 m s^{-1} (IH, 2002).

2.2 Tides

In the Ria Formosa, tides exhibit a semi-diurnal regime and a fortnightly cycle of spring and neap tides. The mean tidal range is 2.0 m, ranging from 0.5 m in neap tides to 3.5 m in spring tides (Melo, 1989), thus a rather intense exchange of water mass occurs during each tide (Sprung, 1994)

and large intertidal areas are exposed to the atmosphere for several hours over each semi-tidal period.

3. Sediment characteristics

The results of grain size analysis of sub-tidal and intertidal superficial sediments are presented in Table 1.1. Near the inlets and in the main channels of the lagoon submitted to strong currents, sandy sediment prevails, while in the inner parts of lagoon and in the intertidal areas predominates mud or muddy-sand sediments (Granja, 1984; Monteiro, 1989).

Table 2.1 – Mean annual values of grain size and calcimetric analysis of superficial sediments in stations located near the inlets/main channels and inner parts of lagoon/intertidal areas (adapted from Monteiro, 1989).

Stations	**Clay** (%)		**Silt** (%)		**Sand** (%)		**$CaCO_3$** (%)	
	Mean	***s.d.***	**Mean**	***s.d.***	**Mean**	***s.d.***	**Mean**	***s.d.***
Inlets / Main channels	0.55	0.39	0.78	0.42	95.58	7.45	5.02	2.34
Inner parts of lagoon / Intertidal areas	6.18	3.70	32.63	16.21	62.10	15.58	7.00	3.26

4 Physico-chemical parameters

4.1 Temperature, dissolved oxygen, pH, salinity, Secchi disk and suspended particulate matter

Water temperature exhibits a seasonal fluctuation with a minimum in winter (13 ºC) and a maximum of 26 ºC in summer (Falcão, 1997; Falcão and Vale, 2003). Concentrations of dissolved oxygen are close to saturation values all over the year (Falcão and Vale, 2003; Newton *et al.*, 2003), probably due to an intense exchange of water between the lagoon and the sea, and to a high primary productivity within the lagoon (Falcão and Vale, 2003). Values of pH vary from 8.0 to 8.6 at neap tides and are slightly lower (≈ 8.2) in spring tides due to mixing with incoming seawater (Falcão and Vale, 2003). Because freshwater inputs to the lagoon are negligible, salinity remains close to 36 (Falcão, 1997), except during sporadic and short periods of winter runoff when it may reach 30 (Falcão *et al*., 1992). The Secchi disk depth ranges annually from 1.5 m to 3.2 m [3], which corresponds to an annual range of light extinction coefficient (k), between 0.5 and 1.1 m^{-1} (Parsons *et al.*, 1984). These low values of k may be explained by the low concentrations (23 g m^{-3}

- 45 g m^{-3}) of suspended particulate matter (SPM) observed over the year. SPM regulates the underwater light climate, and therefore determines the benthic primary productivity in shallow coastal systems (Lorenz *et al*., 1999).

4.2 Nutrients

In the Ria Formosa lagoon, nutrient concentrations exhibit seasonal, spatial and tidal variability (Newton, 1995; Falcão, 1997; Newton *et al.*, 2003).

The average concentrations of dissolved available inorganic nitrogen (DAIN) fluctuate around 20 µM with greater concentrations in the eastern lagoon (10 – 150 µM) compared to the western lagoon (1-35 µM) (Newton *et al.*, 2003). These differences are greater in winter months, with concentrations in the eastern lagoon increasing up to 150 µM, while in the western lagoon DAIN concentrations are around 35 µM. The high levels of DAIN observed in the eastern lagoon during the rainfall period are probably related to the fact that this area is under the impact of rivers (e.g. Gilão), streams (e.g. Almargem) and agricultural runoff.

Phosphate is generally greater than 0.6 µM and always higher in the eastern lagoon (0.75 – 1.4 µM) compared to the western lagoon (0.35 – 1.3 µM). The highest phosphate concentrations are observed during late spring and early summer, decreasing to below 0.8 µM during late summer and early autumn. Phosphate increases during late autumn and early winter, reaching values of 1.2 µM in the eastern lagoon (Newton *et al.*, 2003).

Nitrate and phosphate concentrations also vary with the tide level (Falcão and Vale, 2003). At high tide the concentrations of these nutrients are higher than at low tide, suggesting that they are imported from the sea, mainly during the period of lower water temperatures (Falcão, 1997).

Based on the distribution of DAIN and phosphate concentrations in the lagoon, Newton *et al.* (2003) created GIS maps for the DAIN:P ratio (Fig. 1.5). These maps revealed that during summer DAIN: P ratio is close to the normal Redfield ratio of 16 in the middle of the western lagoon, but is greater than 16 for most of the inner lagoon, suggesting phosphorus limitation. However, near the urban centers of Faro and Olhão and areas adjacent to the seawater inlets, the ratio is less than 16, which points to nitrogen limitation. This is also observed in the far east of the lagoon. During winter, the outer regions close to the seawater inlets of the western lagoon show DAIN:P ratios below the Redfield ratio, while in the inner region of the western lagoon and the entire eastern lagoon there is phosphorus limitation (DAIN:P > 25).

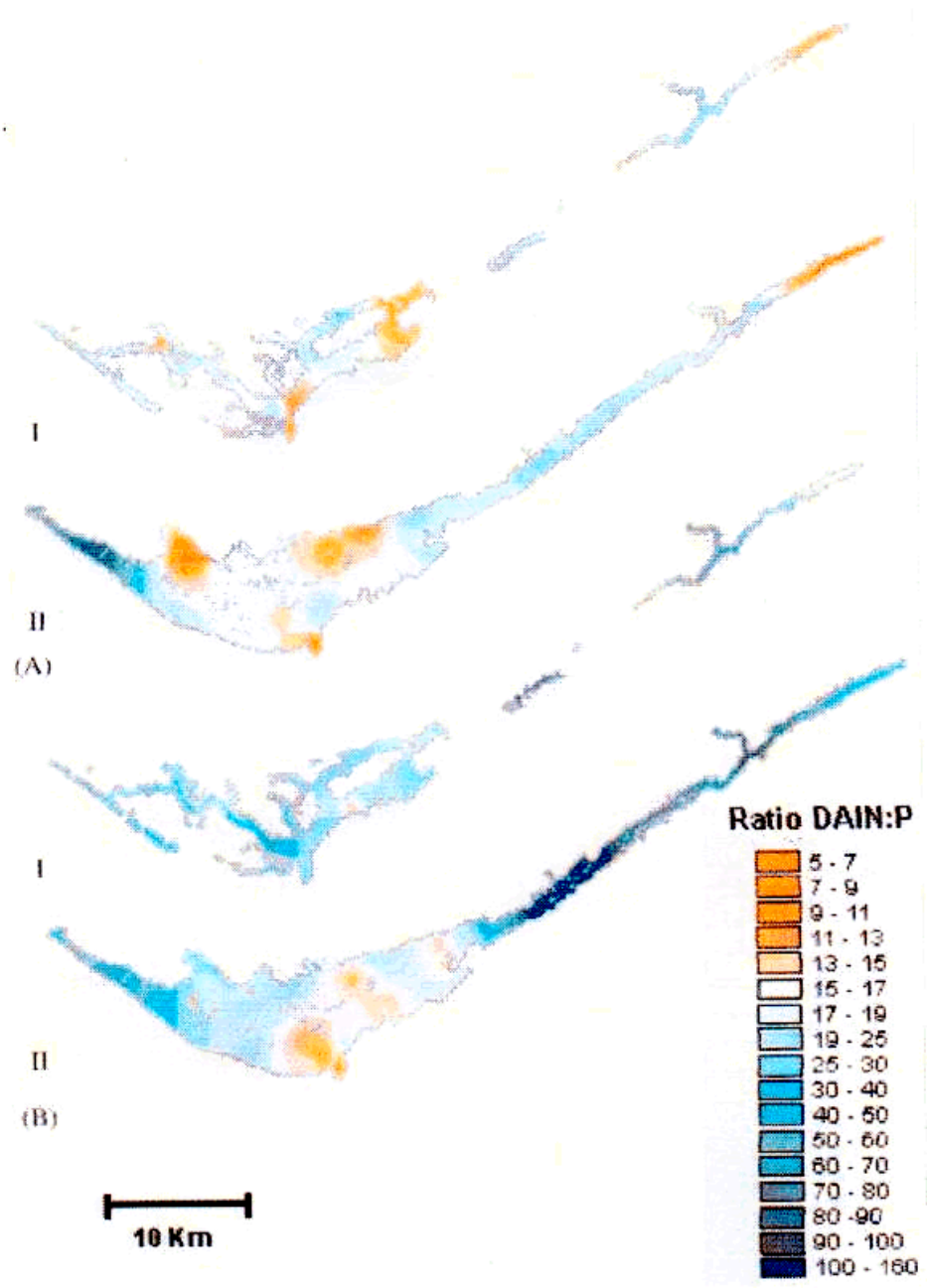

Figure 2.5 – GIS maps of the DAIN:Phosphate ratio in the Ria Formosa lagoon. (A) Summer conditions: (I) low, (II) high water; (b) winter conditions: (I) low, (II) high water (Newton *et al.*, 2003).

5. Vegetation cover

Subtidal and intertidal areas of the lagoon are extensively covered by benthic macrophytes, such as macroalgae (*Enteromorpha* spp. and *Ulva* spp.), seagrasses (*Zostera* sp., *Cymodocea nodosa* and *Ruppia cirrhosa*) and *Spartina maritima* that dominate the low salt marshes (Falcão, 1997).

The intertidal areas are mainly covered by *Spartina maritima* (8 km^2), seagrasses (8.2 km^2) and macroalgal mats (2.5 km^2) (Aníbal, 1998).

5.1 Macroalgae

In the Ria Formosa lagoon, macroalgae occur mainly in the tidal flats. In these areas, 16 species of Phaeophyta (brown algae), 22 species of Chlorophyta (green algae), and 39 species of Rhodophyta (red algae) were identified (Ardré, 1970; Cunha, 1990; Duarte *et al*., 1988; Reis, 1994; Mata, 1997). Of these algae, the most representative are the Ulvales, *Enteromorpha* spp. and *Ulva* spp. (Fig. 1.6), contributing to more than 70 % of the seaweed biomass in the lagoon (Reis, 1994).

a) b)

Figure 2.6 – Macroalgae species in the Ria Formosa lagoon: a) *Ulva lactuca*, b) *Enteromorpha compressa*.

Macroalgae are distributed along a horizontal axis. Substrate, temperature, light intensity, nutrient availability and hydrodynamics (tidal range and currents) are the major factors concerning the algae distribution in this lagoon (Cunha, 1990; Duarte *et al*., 1988).

5.1.1 Spatial distribution

Reis (1994) observed a strong correlation between the Ulvales biomass and substrate, in the eastern part of the lagoon. Intertidal areas of sandy sediments and coarse materials seem to be the more suitable to the establishment of *Enteromorpha* and *Ulva* species. In fact, these algae are a major problem in the growth banks of clams where they have to be constantly removed (Cunha, 1990). However, *Enteromorpha* species are also common in muddy areas (Reis, 1994).

Although *Enteromorpha* and *Ulva* species are frequently observed together (Cunha, 1990; Reis, 1994), it seems that *Enteromorpha* species are dominant in the upper intertidal areas (Cunha, 1990), at a mean depth of 1.2 m (positive above tidal datum). This is probably due to a higher capacity of resistance to extreme environmental conditions (high temperatures, high light

intensities and desiccation) (Pregnall and Rudy, 1985). On the other hand, *Ulva* species colonize mainly subtidal and lower intertidal areas (Cunha, 1990), about 0.8 m above tidal datum.

5.1.2 Seasonal distribution

In the Ria Formosa, macroalgae blooms appeared in September, by the time of the first autumn rainfalls and disappeared gradually during the following spring (Fig. 1.7). Minimum values of seaweed biomass were observed in summer (July-August). This pattern of seasonal variation is typical from transitional areas, between temperate and tropical zones (Morand and Briand, 1996).

Over the year, the mean values of *Enteromorpha* biomasses are higher than for *Ulva* (Aníbal, 1998). This author has documented a minimum *Enteromorpha* biomass in August (4.5 g dw m^{-2}) and a maximum in September (56.1 g dw m^{-2}), while for *Ulva*, biomasses ranged from 1.1 g dw m^{-2} in July-August to 10.2 g dw m^{-2} in February (Fig. 1.7).

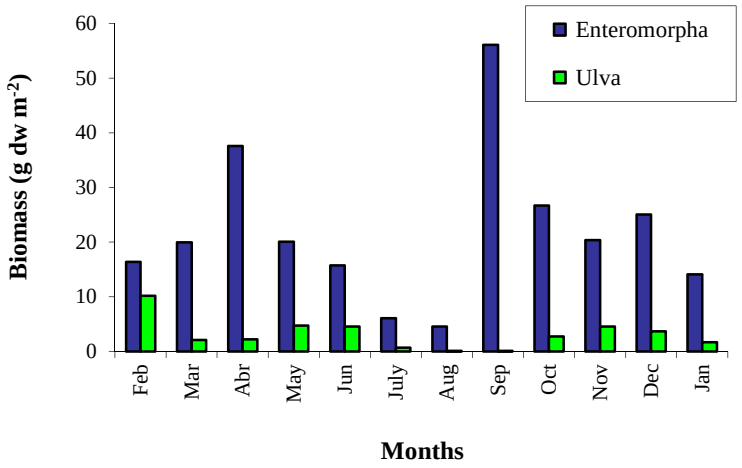

Figure 2.7 - Average monthly biomasses (g dw m^{-2}) of *Enteromorpha* and *Ulva* species in the Ria Formosa lagoon (Adapted from Aníbal, 1998).

CHAPTER III.

Macroalgal primary productivity

1. Introduction

Benthic macroalgae are major contributors to the primary productivity of coastal ecosystems (Mann, 1973; Pregnall and Rudy, 1985; D'Avanzo *et al.*, 1996; Peckol and Rivers, 1996; Kinney and Roman, 1998; Alvera-Azcárate *et al.*, 2003).

Seaweeds productivity is determined by physical (hydrodynamics, light irradiances, temperature), chemical (nutrient availability, salinity, pH) and biological factors, e.g. grazing (Steffensen, 1976; Lapointe and Tenore, 1981; Parsons *et al.*, 1984; Thom and Albright, 1990; Henley *et al*., 1991; Fong *et al.*, 1994; Lobban and Harrison, 1994; Duarte and Ferreira, 1995; Valiela, 1995; Van Den Hoek *et al*., 1995) that may act synergistically or not.

The aim of this chapter was to study the primary productivity of the most representative species of macroalgae (*Enteromorpha* spp. and *Ulva* spp.) in the Ria Formosa lagoon and to evaluate the influence of environmental factors (temperature and light) on the photosynthetic rates of these seaweeds. *Enteromorpha* and *Ulva* photosynthetic rates were studied by generating *P-I* curves for each macroalgae genera.

2. State of the art

In situ (Pregnall and Rudy, 1985; Ferreira and Ramos, 1989; Kinney and Roman, 1998) and laboratory (Arnold and Murray, 1980; Nelson and Siegriest, 1987; Duarte and Ferreira, 1995; Peckol and Rivers, 1996; Hanelt *et al.*, 1997) short-term incubation experiments have been used to determine macroalgal primary productivity.

Methods for the determination of seaweed photosynthetic rates are usually based on measurements of either carbon dioxide consumption or oxygen production per unit time (Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995).

CO_2 is usually measured by the infra-red analysis method or the radiocarbon (^{14}C) method (Van Den Hoek *et al*., 1995). The infra-red analysis method consists in measuring the difference in CO_2 concentration before and after its introduction in the photosynthesis chamber (Van Den Hoek *et al*., 1995). In the radiocarbon method, a high specific-radioactivity isotope ($H^{14}CO_3$) is added to the incubation vessels and the proportion of total radioisotope incorporated into the seaweed is measured (Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995). This method is usually used in oceanic waters because it allows the detection of very low photosynthetic rates. Nevertheless, care

should be taken in interpreting the results obtained by the radiocarbon method because: 1) there is an apparent discrimination in the assimilation rates of the two carbon isotopes ($^{14}CO_2$ and $^{12}CO_2$), 2) the ^{14}C-organic carbon may be lost by exudation during the experiment, 3) some $^{14}CO_2$ fixed may be lost due to dark respiration or photorespiration, which take place simultaneously with photosynthesis (Parsons *et al.*, 1984).

Productivity estimates based on oxygen evolution can be made using an oxygen electrode or the Winkler method (Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995). The advantages of the oxygen methods are that net primary productivity (P_n), gross primary productivity (P_g) and respiration (R) can be directly estimated. However there may be an overestimation of respiration in the light due to photorespiration. Another disadvantage is that heterotrophic respiration is also included in the measurement. Although one order of magnitude less sensitive than the ^{14}C-method, the oxygen methods are suitable for use in coastal waters (Parsons *et al.*, 1984).

The relationship between photosynthesis (P) and light intensity (I) is fundamental to study macroalgal productivity. A general scheme of a *P-I* curve is presented in Fig. 2.1.

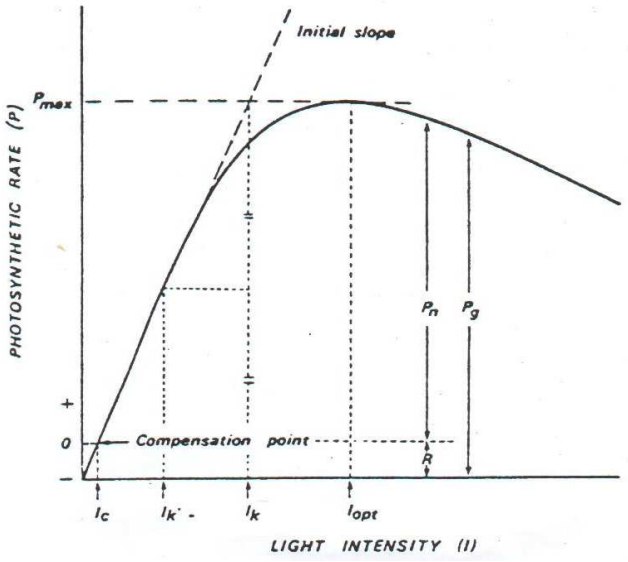

Figure 3.1 - General scheme of a *P-I* curve (Parsons *et al.*, 1984).

This figure shows that an increase in light intensity leads to greater photosynthetic rates up to some asymptotic value, P_{max} (maximum production rate), where photosynthesis becomes light saturated. At this point the producers cannot use any more light because photosynthetic enzymes cannot act fast enough to process light quanta (Parsons *et al.*, 1984).

The initial slope, α, has been defined as the "quantum yield", i.e., the number of moles of oxygen produced (or of carbon incorporated) per unity light intensity. The initial slope is a function of the light reactions and is not usually affected by other environmental factors (Parsons *et al.*, 1984).

I_{opt} is the optimum light intensity for photosynthesis, while, I_k, the saturating irradiance, is defined as the point at which the extrapolated initial slope intercepts P_{max} (Fig. 8). I_k gives a measure of the radiant energy or illumination at light saturation but it does not express photosynthetic efficiency (Parsons *et al.*, 1984; Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995).

When gross photosynthesis (P_g) equals respiration (R), the net photosynthesis (P_n) is zero and the photosynthetic system is at the compensation point (Van Den Hoek *et al.*, 1995). The light intensity at the compensation point is called the compensation light intensity, I_c (Parsons *et al.*, 1984).

If organisms are exposed to a strong light above the point at which they are light saturated, the *P-I* curve may show a decrease in the photosynthetic rates. This phenomenon is termed photoinhibition (Parsons *et al.*, 1984; Valiela, 1995) and it involves damage to some photosystems components (especially PS-II), such as membranes or electron-transport proteins, and changes in the enzymatic activity (Neale and Marra, 1985).

Several mathematical models have been proposed for describing the primary productivity of phytoplankton (Steele, 1962; Jassby and Platt, 1976; Platt *et al.*, 1980; Neale and Marra, 1985; Eilers and Peters, 1988; McBride, 1992; Macedo *et al.*, 1998) and macroalgae (Nelson and Siegrist, 1987; McBride, 1992; Duarte and Ferreira, 1995) as a function of light intensity. The choice of a particular mathematical formulation may lead to different estimates of the *P-I* curve parameters therefore it is important for these formulations to accurately describe experimental data in order to obtain reliable primary productivity estimates.

3. Methodology

3.1 *In situ* incubation experiments

In order to study the seasonal variation of macroalgal primary productivity, *in situ* short-term incubation experiments were carried out during Summer, Autumn, Winter and Spring 2001/2002.

During the incubations, environmental parameters (water temperature, salinity, light, and dissolved oxygen) were also measured in order to evaluate which factors were controlling algal productivity.

Water temperature, salinity and dissolved oxygen concentrations were measured using a multiparameter probe (YSI 55). Measurements of photosynthetically active radiation (PAR) were made underwater and in air, using a LICOR LI-250 light meter.

Macroalgal photosynthetic and respiratory rates were determined by the oxygen method (Thomas, 1988). Three incubation bottles were filled with lagoon water; two (light and dark) were incubated containing macroalgae, and a third without algae (control) was used to assess the effect of planktonic primary production on the experimental results. All experiments were made in triplicate.

Before the experiments, lagoon water samples were collected in order to determine the initial oxygen concentration. These samples were immediately fixed according to the Winkler method (Grasshoff, 1983).

Macroalgal samples were washed to remove sediment deposits, epifauna and attachment materials and immediately incubated, *in situ*, within Winkler bottles of 300 ml.

Incubation times (1 hour) and biomasses (4 to 7 g of fresh weight) were chosen so as to prevent inhibition of photosynthesis by an excess of dissolved oxygen, pH changes or nutrient depletion, and to simultaneously assure that any oxygen changes were detectable (Dromgoole, 1978; Littler, 1979). During incubation, the bottles were rotated at regular intervals to ensure an even exposure to the light. Following the incubation period, water samples for dissolved oxygen determination were immediately fixed and the incubated algae removed from the bottles. Afterwards, algal samples were taken to the laboratory and dried at 80 ºC to determine dry weight (dw).

3.2 Laboratory incubation experiments

Laboratory incubation experiments were performed in order to generate *P-I* curves for both macroalgae genera. As for the *in situ* incubation experiments, photosynthetic and respiratory rates of seaweeds were determined by the oxygen method.

Before the experiments, water samples were collected and filtered in order to determine the initial oxygen and nutrient (ammonium, nitrate, nitrite and phosphate) concentrations.

Macroalgal samples were incubated in the laboratory using light provided by 1500 W tungsten halogen lamps (Drew, 1983), placed 1 m above the samples. Heat produced by the lamps was dissipated using a cold water flow system (Macedo *et al.*, 1998). Light intensity (0 to 1100 μE m^{-2} s^{-1}) was measured by a LICOR LI-250 light meter and attenuation was achieved by means of grey

PVC nets. In order to determine the effect of incubated algal biomass on photosynthetic rates, two Winkler bottles (300 ml) containing 1 g and 4 g fw of algae, respectively, were incubated for each light intensity. The experiments were carried out in triplicate.

Simultaneously with productivity determinations, the effect of light on macroalgal nutrient uptake rates was also studied. After the incubation period (30 minutes), water samples were collected from the incubation bottles in order to determine macroalgal nutrient consumption.

Macroalgal samples were also incubated in the dark in order to determine respiratory rates.

3.3 Sampling analysis

3.3.1 Dissolved oxygen determination

In both *in situ* and laboratory experiments, dissolved oxygen concentrations were determined by the Winkler method (Grasshoff, 1983). All water samples for oxygen determination were analysed in the same day of collection.

3.3.2 Nutrient determination

The ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (HPO_4^{2-}) concentrations were determined using a "SKALAR" autoanalyser according to the methods of Technicon Industrial Systems (Grasshoff, 1983).

3.4 Primary productivity calculations

In the *in situ* incubation experiments, the oxygen variation in the light bottles is a measure of macroalgal and phytoplankton photosynthetic activity ($P_m + P_p$). It is also a function of algae respiration ($R_m + R_p$), bacterial respiration (R_b) and zooplankton respiration (R_z).

$$\text{Light bottle } (\Delta O_2) = P_m + P_p - (R_m + R_p + R_b + R_z)$$

In the dark bottles, the oxygen variation represents the respiration of all the aerobic organisms present.

$$\text{Dark bottle } (\Delta O_2) = R_m + R_p + R_b + R_z$$

Thus, the results will be representative of a community however, for macroalgae, which represent the bulk of the response, the results will reflect the species chosen (Thomas, 1988). In the present study the values concerning plankton productivity and respiration were very similar to initial oxygen values, and were not used in calculations.

In the laboratory experiments, macroalgae are the only organisms contributing to the oxygen variation in either light or dark bottles, because lagoon water was filtered.

The oxygen variation in light bottles is a measure of macroalgal net primary productivity (P_n). Gross primary productivity (P_g) is the sum between P_n and macroalgal respiration (R).

P_n and R were calculated by the following equation:

$$P_n\ (R) = \frac{([O_2]\text{final} - [O_2]\text{initial}) \times V \times F \times Q}{W \times t} \qquad (1)$$

P_n – net primary productivity (mg C g^{-1} dw h^{-1})
R – respiration (mg C g^{-1} dw h^{-1})
$[O_2]$ final – dissolved oxygen concentration at the end of the incubation experiment (mg l^{-1}).
$[O_2]$ initial - dissolved oxygen concentration before the incubation experiment (mg l^{-1}).
V – volume of the incubation bottle (l).
F – conversion factor of oxygen mass to carbon mass (0.29).
Q – photosynthetic/respiratory quotient.
W – macroalgae weight (g dw).
t – incubation time (h).

According to the conventionally accepted stoichiometric equation for photosynthesis (Tian *et al.*, 1993):

$$106\ CO_2 + 16\ NO_3^- + HPO_4^{2-} + 122\ H_2O + 18\ H^+ \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2$$

the oxygen production can be converted to carbon production by a reduction factor of 0.29.
Values of primary productivity expressed as mg O_2 g^{-1} dw h^{-1} were converted to mg C, assuming a photosynthetic quotient of 1.2 (Valiela, 1995). Values of oxygen consumption (or respiration) were converted to carbon equivalents using a respiratory quotient of 1.0 (Thomas, 1988).

3.5 *P-I* curves

In order to generate *P-I* curves, productivity data obtained in the laboratory incubation experiments was fitted to the Steele (1962) and Eilers and Peters (1988) mathematical models because these two mathematical formulations account for photoinhibition.

Author	Model
Steele (1962)	$P = P_{\max} \frac{I}{I_{opt}} e^{\left(1-\frac{I}{I_{opt}}\right)}$
Eilers and Peeters (1988)	$P = \frac{I}{aI^2 + bI + c}$

In Steele's model, the parameters, I_{opt} and P_{max} are directly estimated, while α and I_k were calculated according to the following equations:

$$\alpha = P_{max} \left(\frac{e}{Iopt}\right) \quad (2)$$

$$I_k = \frac{P\max}{S} \quad (3)$$

By differentiating the Eilers and Peters (1988) model, the parameters α, I_{opt}, P_{max} and I_k can be expressed as a function of the *a*, *b* and *c* parameters:

$$\alpha = \frac{1}{c} \quad (4)$$

$$I_{opt} = \sqrt{\frac{c}{a}} \quad (5)$$

$$P_{max} = \frac{1}{b + 2\sqrt{ac}} \quad (6)$$

$$I_k = \frac{c}{b + 2\sqrt{ac}} \quad (7)$$

Macroalgal productivity data were fitted to the mathematical models using non-linear estimation (*Statistica* software version 5.0).

4. Results and Discussion

4.1 *In situ* incubation experiments

4.1.1 Environmental parameters

During the *in situ* productivity incubations, water temperature followed a clear pattern of seasonal variation (Table 2.1), with lower values during the winter period (January-February) and maximum values during spring/summer. Salinity remained close to 36, as expected because the freshwater supply to the lagoon is irrelevant (Falcão, 1997), except during sporadic periods of intense runoff (Falcão *et al*, 1992). Dissolved oxygen concentrations were always close to saturation values, ranging from 6.4 to 9.3 mg l^{-1}. Underwater light irradiances ranged from 404 (February) to 1650 µE m^{-2} s^{-1} (August), and were generally lower than air irradiances due to light attenuation in the water column (Parsons *et al*., 1984).

Table 3.1 - Mean values (± standard deviation) of environmental parameters measured during the *in situ* productivity experiments.

Date	Water temperature (ºC)	Salinity	Dissolved oxygen (mg l^{-1})	PAR (µE m^{-2} s^{-1}) Air	PAR (µE m^{-2} s^{-1}) Underwater
21-08-01	23.6 ± 1.2	35.6 ± 0.1	7.1 ± 0.1	1740 ± 158	1650 ± 150
20-09-01	22.8 ± 0.5	35.6 ± 0.1	6.4 ± 0.1	812 ± 187	781 ± 216
16-10-01	19.8 ± 0.4	35.8 ± 0.1	6.9 ± 0.1	850 ± 167	796 ± 106
16-01-02	13.2 ± 0.8	35.6 ± 0.2	9.3 ± 0.1	1060 ± 82	1256 ± 22
28-02-02	16.6 ± 0.1	35.6 ± 0.1	7.2 ± 0.1	415 ± 10	404 ± 34
27-05-02	23.1 ± 1.0	35.6 ± 0.1	8.0 ± 0.1	1964 ± 20	1502 ± 61

4.1.2 Primary productivity

In the Ria Formosa, macroalgal productivity didn't exhibit a clear pattern of seasonal variation, in contrast to observations in other coastal environments (Pregnall and Rudy, 1985; Kinney and Roman, 1998). Unlike other studies, in which macroalgal photosynthetic rates were higher during

spring-summer (Pregnall and Rudy, 1985; D'Avanzo et al., 1996; Kinney and Roman, 1998), the highest productivity values in the lagoon were observed during autumn and early winter (Fig. 2.2 and 2.3). The maximum net production rates of *Enteromorpha* (2.5 mg C g^{-1} dw h^{-1}) and *Ulva* (6.1 mg C g^{-1} dw h^{-1}) were measured in January, at an ambient water temperature of 13.2 ºC and an irradiance of 1256 µE m^{-2} s^{-1}. The lowest productivity values of *Enteromorpha* (Fig. 2.2) and *Ulva* (Fig. 2.3) were measured in February (16.6 ºC; 415 µE m^{-2} s^{-1}) and May (23.1 ºC; 1964 µE m^{-2} s^{-1}).

Although the photosynthetic rates of both macroalgae genera presented the same pattern of variation, the results show that, for this range of temperatures and light irradiances, *Ulva* (2.1 – 6.1 mg C g^{-1} dw h^{-1}) has consistently higher production rates than *Enteromorpha* (1.2 – 2.5 mg C g^{-1} dw h^{-1}).

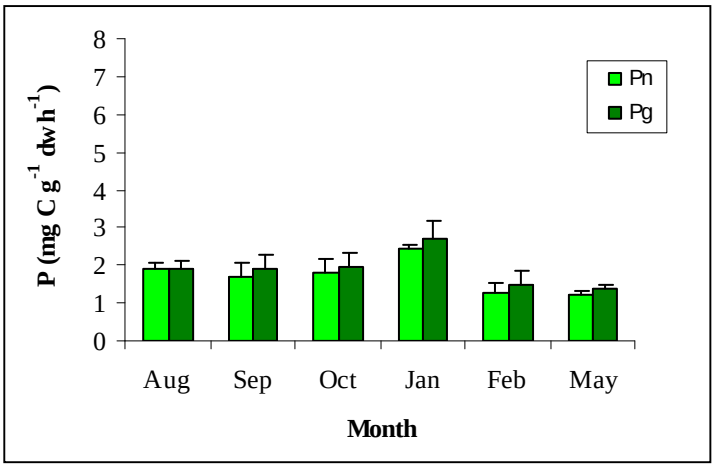

Figure 3.2 - Mean values (± standard deviation) of net (P_n) and gross primary productivity (P_g) for *Enteromorpha* over the experimental period.

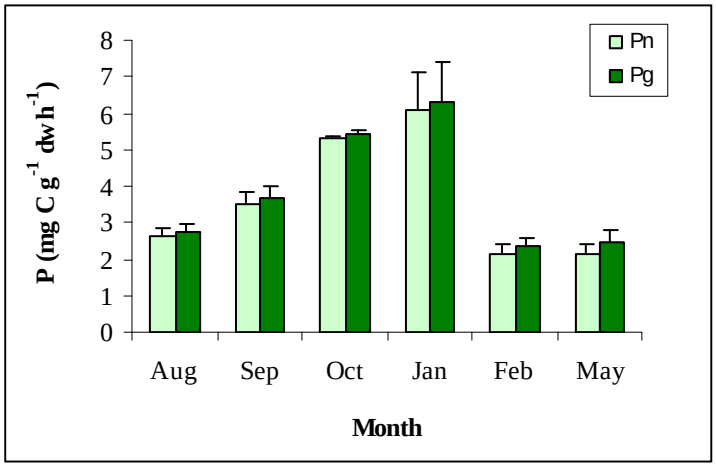

Figure 3.3 – Mean values (± standard deviation) of net (P_n) and gross primary productivity (P_g) for *Ulva* over the experimental period.

In order to understand the variation of *Enteromorpha* and *Ulva* primary productivity, over the experimental period, a regression analysis was performed to evaluate the relationship between environmental factors and photosynthetic rates. This analysis was only performed for the parameters, water temperature and light irradiances because salinity remained relatively constant over the experimental period (Table 2.1) and dissolved oxygen does not determine photosynthesis. Macroalgal productivity was not significantly (p>0.05) related to water temperature (Fig. 2.4) possibly because other environmental factors, such as light or nutrient availability, do not allow the expression of temperature limitation (Rivers and Peckol, 1995).

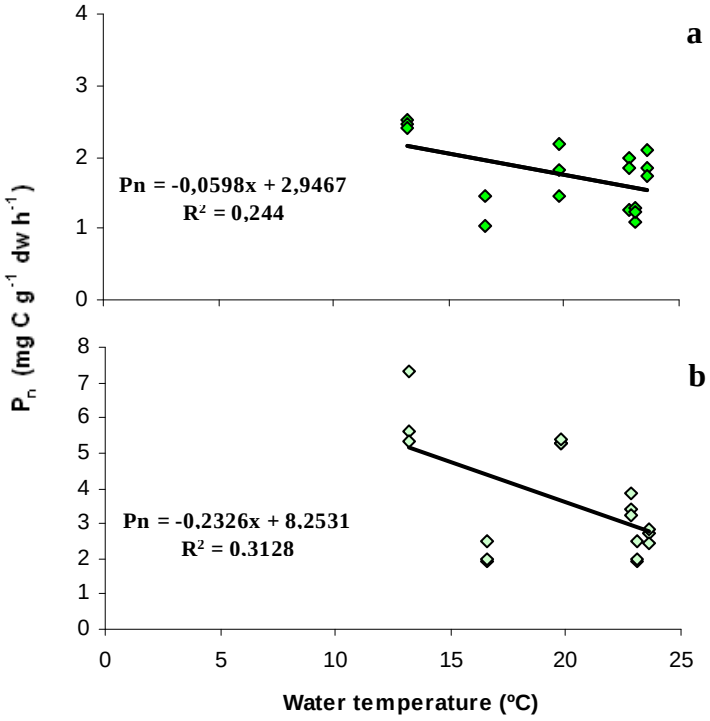

Figure 3.4 - Relationship between water temperature and net primary productivity (P_n) for *Enteromorpha* (a) and *Ulva* (b), with the regression lines and equations.

As shown in Fig. 2.5, light seems to accurately (p<0.05) explain the pattern of variation of seaweed productivity in the Ria Formosa lagoon. Macroalgal productivity increased with light up to a certain irradiance value. At high light irradiances (1740 - 1964 µE m^{-2} s^{-1}), the production rates of both Ulvales were inversely proportional to light, suggesting that the algae were photoinhibited (Parsons *et al*., 1984; Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995).

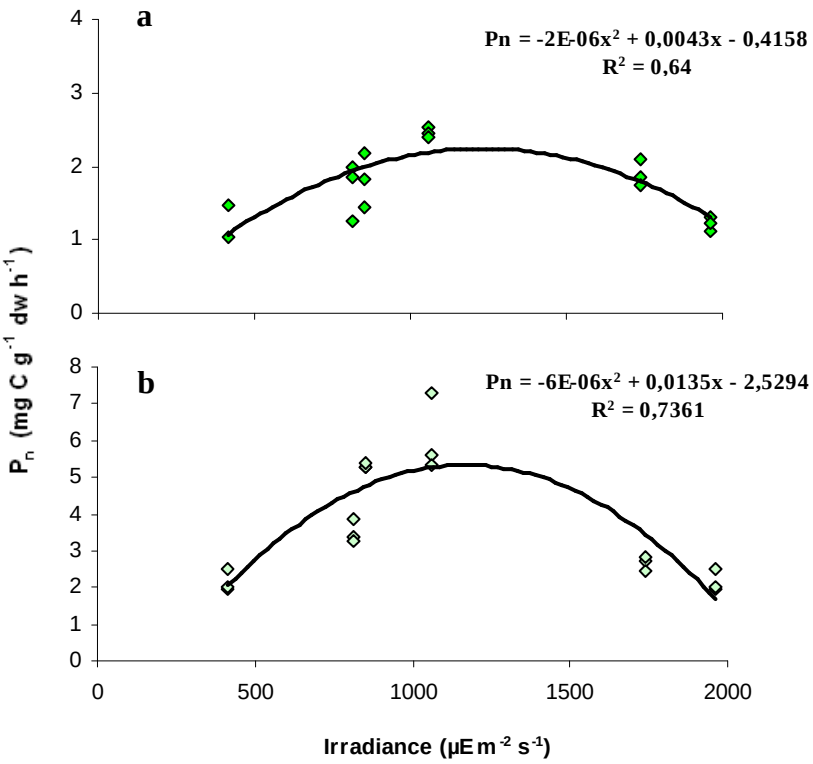

Figure 3.5 - Relationship between light irradiance and net primary productivity (P_n) for *Enteromorpha* (a) and *Ulva* (b), with the regression lines and equations.

4.1.3 Respiratory rates

Macroalgal respiratory rates measured in the *in situ* incubation experiments are presented in Table 2.2. *Enteromorpha* and *Ulva* presented similar respiratory rates, however *Ulva* respiration values (0.08 to 0.35 mg C g^{-1} dw h^{-1}) varied within a broader range than for *Enteromorpha* (0.04 to 0.25 mg C g^{-1} dw h^{-1}).

Table 3.2 - Mean (± standard deviation) respiratory rates of *Enteromorpha* and *Ulva* measured in the i*n situ* incubation experiments.

Date	**Respiratory rates** (mg C g^{-1} dw h^{-1})	
	Enteromorpha spp.	*Ulva* spp.
21-08-01	0.04 ± 0.01	0.08 ± 0.06
20-09-01	0.21 ± 0.02	0.18 ± 0.02
16-10-01	0.13 ± 0.02	0.10 ± 0.09
16-01-02	0.23 ± 0.05	0.21 ± 0.01
28-02-02	0.25 ± 0.06	0.22 ± 0.17
27-05-02	0.20 ± 0.04	0.35 ± 0.04

4.2 Laboratory incubations experiments

In the laboratory, incubation experiments were performed for different light irradiances in order to generate *P-I* curves for each macroalgae genera.

4.2.1 Primary productivity

Net productivity values of *Enteromorpha* (Table 2.3) and *Ulva* (Table 2.4) varied with the incubated biomass. Correlation analysis revealed that, for both genera, production rates were significantly lower ($p < 0.01$) in the incubation bottles containing higher alga biomasses (4g fw). This may have happened because the use of freshly cut pieces of alga thallus normally generates an increase in thallus respiration due to tissue damage (Drew, 1983; Lobban and Harrison, 1994), and consequently photorespiration is overestimated and net photosynthesis underestimated (Parsons *et al.*, 1984). The productivity results used to generate *P-I* curves were the ones concerning to the experiments in which 1g fw of algal biomass was incubated.

Independently from the incubated biomass, the photosynthetic rates of both macroalgae species exhibited a similar pattern of variation with light. Seaweed productivity values increased with increasing irradiances, but at high light levels (1100 $\mu E\ m^{-2}\ s^{-1}$), production rates were reduced probably due to photoinhibition (Parsons *et al.*, 1984; Lobban and Harrison, 1994; Van Den Hoek *et al*., 1995), as observed in the *in situ* incubation experiments. In the laboratory, *Enteromorpha* photosynthetic rates (Table 4) were higher than the *Ulva* photosynthetic rates (Table 5), unlike what was observed in the *in situ* experiments in which *Ulva* presented the highest productivity values.

Table 3.3 – Average net primary productivity (P_n) of *Enteromorpha* spp. for each light incubation experiment. **C.V.** – variation coefficient.

Irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*Enteromorpha* $\boldsymbol{P_n}$			
	Incubated biomass (g dw)		**Incubated biomass** (g dw)	
	0.12 ($\Leftrightarrow$ 1 g fw)	**C.V.** (%)	***0.48*** ($\Leftrightarrow$ 4 g fw)	**C.V.** (%)
70	2.8	*6.3*	0.4	*63.6*
150	7.1	*11.3*	2.5	*3.1*
300	7.1	*1.2*	2.3	*13.9*
500	8.5	*0.3*	3.7	*2.5*
850	9.2	*2.2*	3.9	*3.0*
1100	1.8	*25.0*	0.1	*4.9*

Table 3.4 – Average net primary productivity (P_n) of *Ulva* spp. for each light incubation experiment. **C.V.** – variation coefficient.

Irradiance ($\mu E\ m^{-2}\ s^{-1}$)	*Ulva* $\boldsymbol{P_n}$			
	Incubated biomass (g dw)		**Incubated biomass** (g dw)	
	0.18 ($\Leftrightarrow$ 1 g fw)	**C.V.** (%)	***0.67*** ($\Leftrightarrow$ 4 g fw)	**C.V.** (%)
70	0.5	*30.4*	0.3	*23.5*
150	5.1	*37.2*	1.4	*4.3*
300	3.8	*3.2*	1.5	*7.7*
500	4.9	*2.5*	1.7	*5.8*
850	7.0	*4.1*	2.9	*1.4*
1100	1.0	*42.0*	0.2	*55.3*

Comparing both incubation experiments one can notice that, for a similar value of incubated biomass (4 g fw) and at the same range of light irradiances (415 – 850 $\mu E\ m^{-2}\ s^{-1}$) the *Enteromorpha* productivity values measured in the laboratory (2.3 – 3.9 mg C g^{-1} dw h^{-1}) were higher than those measured *in situ* (0.76 – 1.67 mg C g^{-1} dw h^{-1}). Conversely, *Ulva* production rates were higher in the *in situ* incubation experiments (3.42 – 4.64 mg C g^{-1} dw h^{-1}) than in the laboratory (1.7 - 2.9 mg C g^{-1} dw h^{-1}). These results may be explained by differences in the experimental conditions (water temperature and nutrient concentrations) or in the algae physiological state at the time of the experiments (Fong *et al*., 1994).

4.2.2 Respiratory rates

The seaweed respiratory rates measured in the laboratory are presented in Table 2.5. Correlation analysis revealed that algae respiration was significantly (p<0.01) influenced by the incubated biomass.

Despite the incubated biomass, *Enteromorpha* (1.2 mg C g^{-1} dw h^{-1}) and *Ulva* (1.4 mg C g^{-1} dw h^{-1}) showed similar respiratory rates.

Once again, laboratory and field experiments yielded different results, probably due to different experimental conditions.

Table 3.5 – Mean respiratory rates of *Enteromorpha* and *Ulva* with different incubated biomasses. **C.V.** – variation coefficient.

	Respiratory rates (mg C g^{-1} dw h^{-1})			
	Enteromorpha spp.		*Ulva* spp.	
	Incubated biomass (g dw)		**Incubated biomass** (g dw)	
	0.12	***0.48***	***0.18***	***0.67***
Average	1.2	0.8	1.4	0.8
C.V. (%)	*12.9*	*10.0*	*0.7*	*1.2*

4.2.3 *P-I* curves

Because Steele's (1962) model did not accurately describe the experimental data (p = 1), only the results concerning the Eilers and Peters (1988) rational model will be presented and discussed.

P-I curves for *Enteromorpha* and *Ulva* are presented in Figures 2.6 and 2.7, respectively.

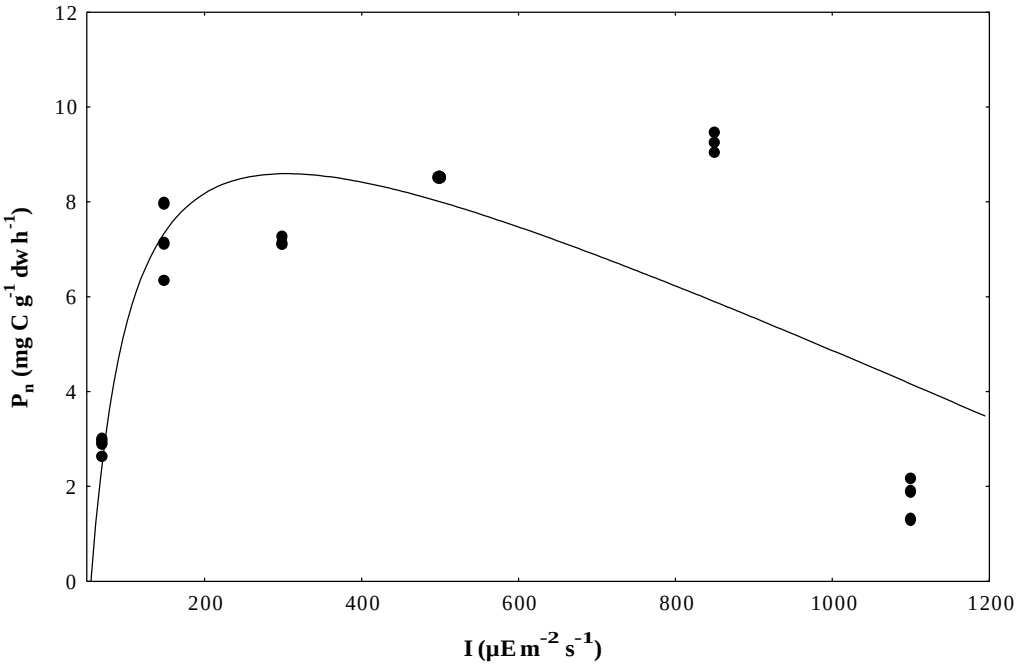

Figure 3.6 – *Enteromorpha* spp. *P-I* curve. Filled circles represent the results obtained in the laboratory incubation experiments.

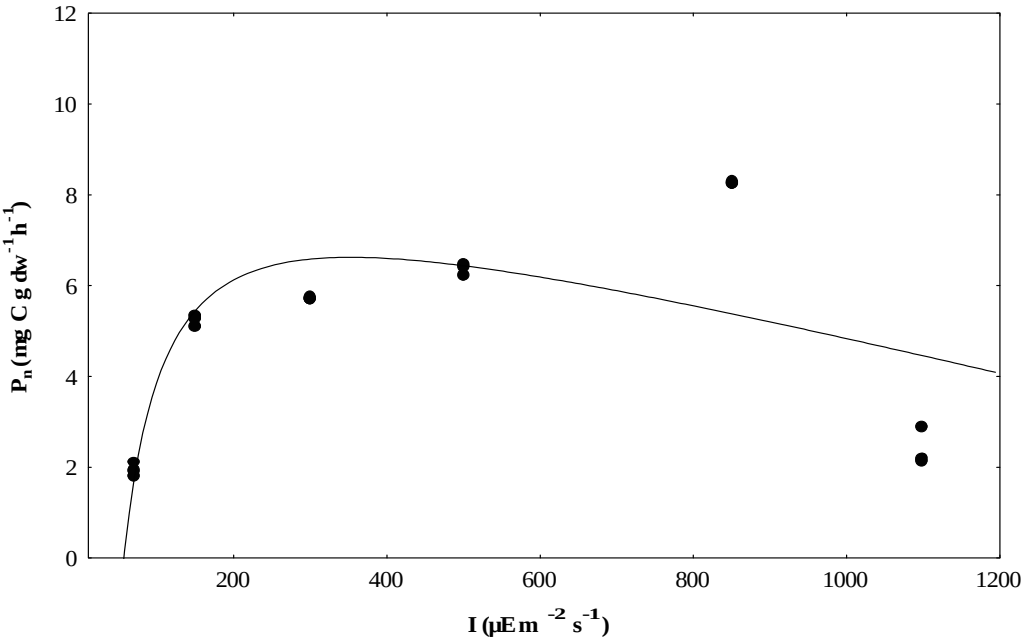

Figure 3.7 – *Ulva* spp. *P-I* curve. Filled circles represent the results obtained in the laboratory incubation experiments.

The *P-I* curves parameters estimated for *Enteromorpha* and *Ulva* are presented in Table 2.6.

Table 3.6 – *Enteromorpha* spp. and *Ulva* spp. *P-I* curves parameters.

***P-I* curves parameters**	*Enteromorpha* spp.	*Ulva* spp.
α	0.08	0.10
P_{max}	3.55	3.66
I_{opt}	97	72
I_k	47	35
p	<0.01	<0.05

The initial slopes (α) estimated for *Enteromorpha* (0.08) and *Ulva* (0.1) were very similar, suggesting comparable photosynthetic performances (Arnold and Murray, 1980). The steep slopes that characterize sheet-like (*Ulva*) and tubular seaweeds (*Enteromorpha*) indicate high photosynthetic performances (Arnold and Murray, 1980).

The maximum production value (P_{max}) estimated for *Ulva* (3.66 mg C g^{-1} dw h^{-1}) was slightly higher than the P_{max} estimated for *Enteromorpha* (3.55 mg C g^{-1} dw h^{-1}), unlike what was visually observed in the laboratory experiments in which *Enteromorpha* presented the highest photosynthetic rates.

Similar I_{opt} values were estimated for *Enteromorpha* (47 µE m^{-2} s^{-1}) and *Ulva* (35 µE m^{-2} s^{-1}), suggesting similar responses to light (Van Den Hoek *et al*., 1995). The I_k estimated for these seaweeds were close to the values referred by Arnold and Murray (1980) for subllitoral species of *Enteromorpha intestinalis* (55.8 µE m^{-2} s^{-1}), *Ulva lobata* (75.5µE m^{-2} s^{-1}) and *Ulva rigida* (50.3 µE m^{-2} s^{-1}), but lower than the I_k values (> 500 µE m^{-2} s^{-1}) observed by other authors (Van Den Hoek *et al*., 1995) for intertidal macroalgae species. Pregnall and Rudy (1985) also estimated higher I_k values (130 µE m^{-2} s^{-1}) for *Enteromorpha* spp., while experimental work carried out in the Tagus estuary (Ferreira and Ramos, 1989) gave a saturation light intensity value of 326.4 Wm^{-2} (≈ 1500 µE m^{-2} s^{-1}) for *Ulva lactuca*.

4.2.4 Nutrient uptake rates

In the laboratory incubation experiments, macroalgal nutrient uptake rates were simultaneously determined with the productivity rates in order to evaluate the effect of light on seaweeds nutrient consumption. Ammonium (NH_4^+), nitrate (NO_3^{2-}), nitrite (NO_2^-) and phosphate (HPO_4^{2-}) uptake rates of *Enteromorpha* and *Ulva* at different irradiances are presented in Table 2.7. The nutrient uptake rates of both Ulvales were highly variable with light. For some nutrients, especially for NO_2^-

and HPO_4^{2-}, negative uptake rates were observed, meaning that nutrient release occurred instead of nutrient consumption.

Table 3.7 – Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (HPO_4^{2-}) uptake rates for *Enteromorpha* and *Ulva*, at different irradiances.

Irradiances ($\mu E\ m^{-2}\ s^{-1}$)	**Nutrient Uptake Rates ($\mu M\ g^{-1}\ fw\ h^{-1}$)**							
	Enteromorpha spp.				*Ulva* spp.			
	NH_4^+	**NO_3^-**	**NO_2^-**	**HPO_4^{2-}**	**NH_4^+**	**NO_3^-**	**NO_2^-**	**HPO_4^{2-}**
70	1.02	3.80	0.02	- 0.86	1.16	2.24	0.02	- 0.68
150	7.60	11.66	0.00	- 1.72	12.94	4.14	- 0.00	- 0.06
300	2.42	1.06	0.02	0.54	0.24	0.26	- 0.04	0.22
500	3.36	5.70	0.08	0.42	13.06	2.68	- 0.02	- 0.46
850	1.30	1.70	- 0.02	- 0.10	0.10	1.38	- 0.04	- 0.54
1100	- 1.30	5.00	- 0.06	- 4.76	2.18	- 0.40	- 3.84	- 0.88

The results of the regression analysis between macroalgal nutrient uptake rates and light irradiances are presented in Tables 2.8 and 2.9. No significant relationship (p>0.05) was found between light and macroalgal nutrient uptake rates, unlike what was observed by Lapointe and Tenore (1981) for *Ulva fasciata*. According to these authors, the uptake of NO_3^{2-} by *Ulva fasciata* was inversely proportional to light intensity and it was mostly dependent on the nutrient water concentrations. Other authors (Van Den Hoek *et al.*, 1995) also referred that light can influence nutrient uptake through photosynthesis. For example, in stimulating the algae growth, light indirectly increases the nutrient uptake. However, opportunistic macroalgae, such as *Enteromorpha* and *Ulva* are known for having "luxury" consumption (Fong *et al.*, 1994), which means that they are always taking up nutrients until a maximum storage capacity is achieved, thus it is not surprising that no relationship between seaweed nutrient uptake rates and light was observed in the present work.

Table 3.8 – Relationship between *Enteromorpha* nutrient uptake rates and light. In the linear regressions, the nutrient uptake rate (µM g^{-1} fw h^{-1}) is the dependent variable (y) and irradiance is the independent variable (x).

Nutrient	Regression equations	R^2	*p*-value
NH_4^+	y = -0.0015x + 1.634	0.14	$p > 0.05$
NO_3^{2-}	y = -0.1428x + 4.903	0.35	$p > 0.05$
NO_2^-	y = -0.0014x + 0.377	0.09	$p > 0.05$
HPO_4^{2-}	y = -0.0012x + 0.051	0.11	$p > 0.05$

Table 3.9 – Relationship between *Ulva* nutrient uptake rates and light. In the linear regressions, the nutrient uptake rate (µM g^{-1} fw h^{-1}) is the dependent variable (y) and irradiance is the independent variable (x).

Nutrient	Regression equations	R^2	*p*-value
NH_4^+	y = -0.0016x + 1.650	0.11	$p > 0.05$
NO_3^{2-}	y = -0.0012x + 1.454	0.26	$p > 0.05$
NO_2^-	y = -0.00003x + 0.013	0.23	$p > 0.05$
HPO_4^{2-}	y = -0.0003x - 0.060	0.04	$p > 0.05$

4.3. Comparison with other studies

The macroalgal photosynthetic rates measured in the incubation experiments (*in situ* and laboratory) ranged from 0.1 to 9.2 mg C g^{-1} dw h^{-1} for *Enteromorpha* spp, and from 0.2 to 7.0 mg C g^{-1} dw h^{-1} for *Ulva* spp.. These productivity values are comparable to data obtained by other authors on these macroalgae species. For example, *Enteromorpha compressa* net productivity values ranging from 0.02 to 10.8 mg C g^{-1} dw h^{-1} were referred by Kautsky (1982). *In situ* incubation experiments with *Enteromorpha* spp. yielded net production values varying between 0.88 and 5.00 mg C g^{-1} dw h^{-1} (Kinney & Roman, 1998), while other authors (Arnold & Murray, 1980) measured a maximum value of 7.3 mg C g^{-1} dw h^{-1} in laboratory experiments with *Enteromorpha intestinalis*. *In situ* productivity incubations carried out in the Tagus Estuary (Ferreira, 1989) yielded lower net primary productivity values for *Ulva lactuca* (0.06 to 1.92 mg C g^{-1} dw h^{-1}) than those observed in the present study (0.2 and 7.0 mg C g^{-1} dw h^{-1}). According to Valiela (1995), at the range of light intensities registered in the present work, the primary productivity of *Ulva* spp. ranged from 4 to 9 mg C g^{-1} dw h^{-1}. Highest photosynthetic rates of 6.5 mg C g^{-1} dw h^{-1} and 9.2 mg C g^{-1} dw h^{-1} were referred by Arnold and Murray (1980) for *Ulva rigida* and *Ulva lobata*, respectively.

The dark respiratory rates of *Enteromorpha* spp. measured in the *in situ* incubation experiments (0.04 – 0.25 mg C g^{-1} dw h^{-1}) are comparable to those referred by other authors (Van Den Hoek *et al.*, 1995) for *Enteromorpha intestinalis* (0.18 mg C g^{-1} dw h^{-1}) and *Enteromorpha linza* (0.18 mg C g^{-1} dw h^{-1}). The *Ulva* respiration values observed in the *in situ* incubation experiments (0.08 –0.35 mg C g^{-1} dw h^{-1}) were also similar to the values referred by other authors (Ferreira, 1989; Van Den Hoek *et al.*, 1995) for *Ulva lactuca*. These authors referred values of 0.16 and 0.27 mg C g^{-1} dw h^{-1} for this seaweed specie. Conversely, the *Enteromorpha* (0.8 – 1.2 mg C g^{-1} dw h^{-1}) and Ulva (0.8 – 1.4 mg C g^{-1} dw h^{-1}) respiratory rates measured in the laboratory incubation experiments were higher than the ones referred above.

CHAPTER IV.

Macroalgal model

1. Introduction

Ecological models are useful instruments for the analysis and forecasting of an ecological problem (Jørgensen, 1994). The fact that models are fast and relatively easy to use gives them an advantage in relation to field and experimental work because it takes less time and money to get the same information. Nevertheless, field and experimental work are crucial for model calibration and validation.

Models to evaluate macroalgal productivity are not common in the literature despite its contribution to carbon fixation and nutrient removal in coastal ecosystems. A lot has been done in simulating *Ulva rigida* growth (Coffaro and Sfriso, 1997; Solidoro *et al*., 1997a; Solidoro *et al*., 1997b) because of the effects of these macroalgal blooms in the Venice lagoon. There is also some work (Alami, 2000) on simulating the annual variation of brown algae (*Halopteris scoparia*, *Dictyota dichotoma*, *Cystoseira balearica*) biomass in the Bay of Calvi. In order to estimate the sustainable yield of the red algae, *Gelidium sesquipedale*, Duarte and Ferreira (1997) developed a model to simulate the population dynamics and productivity of this alga by combining demographic models with biomass density models. In the present work, macroalgal productivity was evaluated using a model that simulates the biomass density dynamics. In this type of models, the population biomass is expressed as a whole (frequently as a carbon pool) without discriminating between size or life history phases. These models are often employed in primary productivity calculations (Seip *et* al., 1979; Ferreira and Ramos, 1989; Duarte and Ferreira, 1993; Alvera-Azcárate, 2003).

The objective of setting up the present model was to simulate the annual productivity of the Ulvales (*Enteromorpha* spp. and *Ulva* spp.) in the Ria Formosa, in order to understand their contribution to the overall ecosystem production.

2. Description of the model

2.1 Background

In the Ria Formosa, *Enteromorpha* and *Ulva* primary productivity was higher during the autumn/early winter period, in agreement with the pattern of biomass variation described by Aníbal (1998). According to the results of the *in situ* incubation experiments, the high availability of PAR (below photoinhibition values) recorded during this period may explain the high values of seaweed productivity and biomass observed in the lagoon. During the winter season, there seems to be

phosphorus limitation in most part of the lagoon (Falcão, 1997; Newton *et al.*, 2003), suggesting that its availability does not limit macroalgal productivity, as referred by other authors (Lowthion and Soulsby, 1985; Sfriso *et al.*, 1989). These findings may be due to the ability of opportunistic macroalgae, such as *Enteromorpha* and *Ulva*, to store the surplus of nutrients, also known as "luxury consumption" (Kuhl, 1962; Waite and Mitchell, 1972; Lapointe and Tenore, 1981; Rosenberg and Ramus, 1982; Fujita, 1985; Fong *et al.*, 1994). Aside from enabling algae survival at low nutrient concentrations and even in the absence of the nutrient, the "luxury consumption" gives them a competitive advantage relatively to other primary producers, such as phytoplankton and other benthic macroalgae. In fact, the phytoplanktonic productivity in the lagoon is characterised by lower values in winter (Falcão and Vale, 2003). During summer, there is also nutrient (N and P) limitation in the lagoon (Newton *et al.*, 2003), however the high light irradiance (above photoinhibition values) recorded during this season are probably the environmental factor responsible for the low values of seaweed productivity and biomass. Conversely, the highest values of phytoplanktonic productivity are measured during the spring/summer period.

2.2 Choice of forcing functions

The forcing functions normally used for modelling primary productivity are light, water temperature and dissolved nutrients (N and P) concentrations (Ferreira and Ramos, 1989; Duarte, 1995; Duarte and Ferreira, 1997; Solidoro *et al.*, 1997b; Chapelle *et al.*, 2000).

According to the results obtained in the previous chapter, no relationship was found for water temperature and macroalgal primary productivity in the Ria Formosa. Thus, this parameter was not considered as a forcing function.

Several studies (Falcão, 1997; Newton *et al*., 2003) revealed that there is spatial and seasonal nutrient (N and P) limitation in the lagoon. Thus, nutrient (dissolved inorganic nitrogen and phosphorus) concentrations were considered as forcing functions.

Because seaweed productivity in this ecosystem is limited by light, this parameter was chosen as a forcing function of the model.

2.3. Model development

As already mentioned, opportunistic macroalgae have luxury consumption; therefore the processes of photosynthesis and nutrient uptake from the water are separated and can be modelled by only taking into account the tissue concentration of nitrogen and phosphorus.

The state variables of the model are: biomass (*B*), internal nitrogen (*N*) and internal phosphorus (*P*) concentrations.

The dynamics of seaweed biomass can be considered as governed by the following processes: gross primary productivity, respiration and mortality.
A general differential equation for biomass *B* that includes these processes is:

$$\frac{dB}{dt} = (P_g - R - M)B \quad (8)$$

where *B* (g dw m^{-2}) is the biomass for each seaweed at time *t*, P_g (mg C g dw^{-1} h^{-1}) is gross primary productivity, *R* (mg C g dw^{-1} h^{-1}) is the respiration rate, and *M* is the natural mortality rate.

2.3.1 Gross Primary Productivity

Gross primary production, P_g, was calculated as following:

$$P_g = P_{max} \times f(I) \times f(N, P) \quad (9)$$

where P_{max} is the maximum photosynthetic rate for each macroalgae, *I* is the light intensity, *N* and *P* correspond to the nitrogen and phosphorus internal concentrations, and *f(I), f(N, P)* are the limiting functions for light and nutrients.

2.3.1.1 Forcing functions and limitations

2.3.1.1.1 Light

2.3.1.1.1.1 Light at the surface of the water

Light energy available at the water surface (I_0) was calculated according to Brock (1981), considering the annual light energy variation, the variation of the daily photoperiod, and the Photosynthetically Active Radiation (PAR).

Light intensity over the year varies as a sinusoidal function, with a minimum intensity in winter and a maximum in summer, as shown in Figure 3.1. The main factors that affect these changes are

the declination of Earth, solar altitude, the hour of sunrise and sunset, the transmission of light through the atmosphere, and the albedo (the fraction of light reflected by the water surface) that for water is considered to be of 6 %.

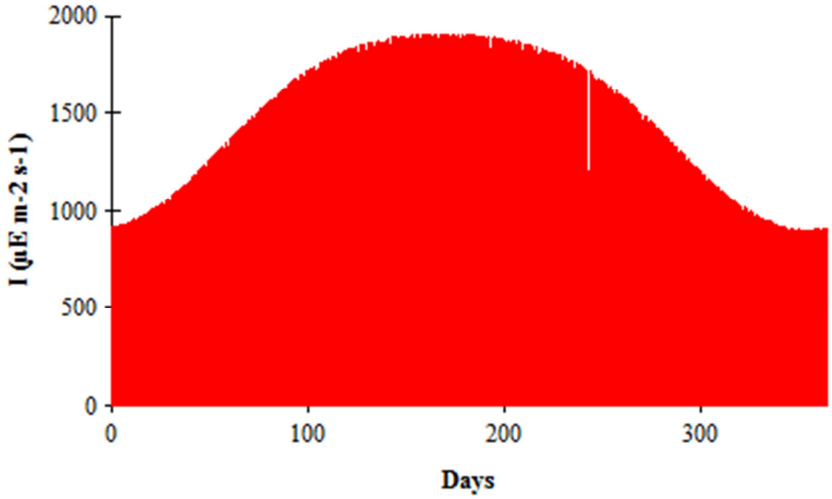

Figure 4.1 – Annual variation of light intensity (I, µE m^{-2} s^{-1}).

The photoperiod also follows a sinusoidal function, with an intensity of zero during night hours and a maximum during the day. The photoperiod is also longer in summer days than in winter days (Fig. 3.2).

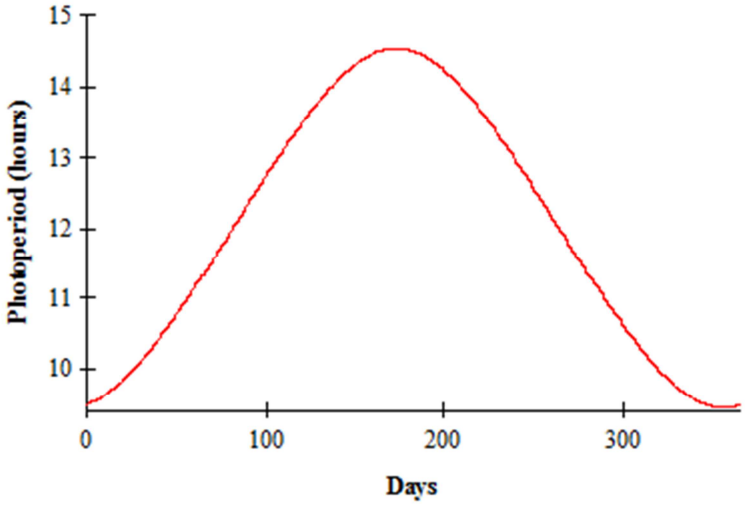

Figure 4.2 – Annual variation of photoperiod.

Of the total amount of light that arrives at the surface, only PAR is usable for photosynthesis. The PAR was considered to be 42 % of the total available light.

2.3.1.1.1.2 Light at the bottom

The light that reaches the bottom was calculated by the Lambert-Beer equation (Parsons *et al*., 1984; Mann & Lazier, 1991; Valiela, 1995):

$$I_z = I_0 \, e^{-kz} \qquad (10)$$

where I_z (µE m^{-2} s^{-1}) is the light available for photosynthesis at the depth z (m), I_0 (µE m^{-2} s^{-1}) is the light available at the water surface, and k (m^{-1}) is the light extinction coefficient. In the present model, k was considered constant with a value of 0.8 m^{-1}, which corresponds to the mean annual value (0.5 - 1.1 m^{-1}) of this parameter in the Ria Formosa.

The depth, z, was determined from the tidal cycle simulation of the water level and the average height of the macrophyte stand above datum (h), such that:

$$z = \text{Tidal height} - h \qquad (11)$$

z is different for each macroalgae, because zonation depends on bathymetry and resistance to emersion. The average height of *Enteromorpha* stand above datum is 1.2 m, while *Ulva* colonizes areas 0.8 m above tidal datum. Thus, the quantity of light available for these seaweeds is different and must therefore be treated separately.

2.3.1.1.1.3. Tide simulation

In mesotidal lagoons, tides establish the regime of emersion-immersion periods for intertidal species. According to some authors, seaweed productivity occurs at a reduced rate when the algae are air exposed (Lowthion and Soulsby, 1985) compared to when they are immersed. In the present model, primary productivity was considered to be null during emersion periods.

The sinusoidal pattern of tidal height was simulated according to equation 12 (adapted from Ferreira and Ramos, 1989) using a time step of one hour.

$$Tidal\ height = \frac{(HT + LT)}{2} + \frac{(HT - LT)}{2} \times \cos\left(\frac{\pi t}{tideperiod}\right) \qquad (12)$$

where *t* is the time and *tide period* is the time interval between a high tide and the subsequent low tide. In the Ria Formosa lagoon the tide period is of 6.25 hours corresponding to a semi-diurnal tidal regime. The water levels (m) at hight tide (HT) and low tide (LT) were calculated by the following equations:

$$HT = \frac{(\max HT + \min HT)}{2} + \frac{(\max HT - \min HT)}{2} \times \cos\left(\frac{\pi t}{lunarcycle}\right) \quad (13)$$

$$LT = \frac{(\max LT + \min LT)}{2} + \frac{(\max LT - \min LT)}{2} \times \sin\left(\frac{\pi t}{lunarcycle}\right) \quad (14)$$

where *max HT* and *min HT* are the maximum and minimum water levels for high tides, *max LT* and *min LT* are the maximum and minimum water levels for low tides, *t* is the time and *lunar cycle* is the time interval between a spring tide and a neap tide (7 days) (Fig. 3.3). The values of *max HT*, *min HT*, *max LT* and *min LT* were determined from data on tidal heights (IH, 2002) for the period of fieldwork.

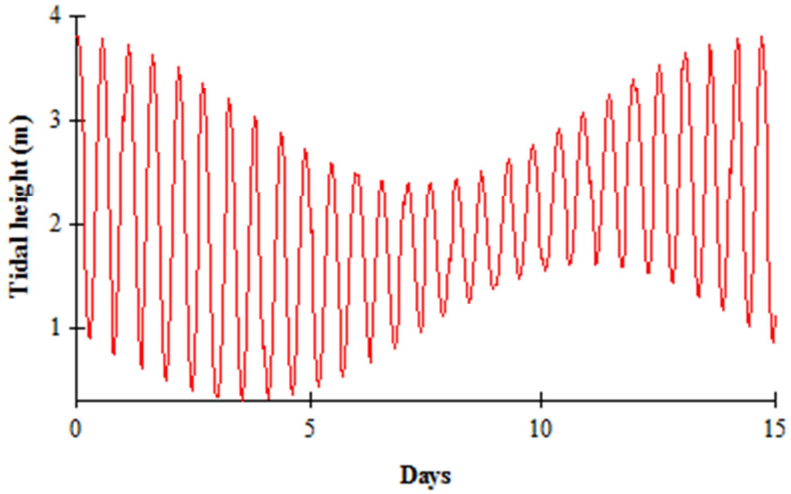

Figure 4.3 – Tidal height over a spring/neap tidal cycle.

2.3.1.1.1.4. Light limitation

In the *in situ* and laboratory incubation experiments, *Enteromorpha* and *Ulva* exhibited photoinhibition. Thus, a light function including photoinhibition (Steele, 1962) was selected to simulate light limitation, according to the following equation:

$$f(I) = \frac{I}{I_{opt}} e^{1-\frac{I}{Iopt}} \quad (15)$$

where I (µE m^{-2} s^{-1}) is the light at the surface of the macroalgae and I_{opt} (µE m^{-2} s^{-1}) is the optimum light level for each macroalgae species.

2.3.1.1.2 Nutrients

Data on dissolved nutrients concentrations were obtained from the monthly values measured by Newton (1995) and Falcão (1997). In order to have a complete time series for a year it was necessary to interpolate these data.

In the work developed by Waite and Mitchell (1972) and Steffensen (1976) for *Ulva lactuca*, there seems to be no distinction between the ammonium and nitrate uptake by this algae. Other authors (Van Den Hoek *et al.*, 1995) also stated that some opportunistic seaweed, like *Codium*, utilize more than one form of nitrogen giving them a competitive advantage over other algae lacking this capacity. Thus, in the present model dissolved inorganic nitrogen (DIN) was used instead of separate nitrogen forms (Fig. 3.4). The main form of phosphorus used by seaweeds is ortophosphate (Van Den Hoek *et al.*, 1995). Phosphate concentrations used in the model are presented in Figure 3.5.

The use of a nutrient limitation in a macroalgal model is not common because there is no direct influence of the available nutrient quantity on primary productivity. Opportunistic macroalgae, such as *Enteromorpha* and *Ulva*, can store the nutrients they uptake from the water in their cells (Lapointe and Tenore, 1981; Rosemberg and Ramus, 1982; Fujita, 1985; Fong *et al*., 1994). According to other studies (Coffaro and Sfriso, 1997; Solidoro *et al.*, 1997b), nutrient limitation was based on the internal concentration of nitrogen and phosphorus.

The equations for internal nitrogen and internal phosphorus concentrations (Solidoro *et al.*, 1997b) are:

$$\frac{dN}{dt} = Nup \times Nfb - PgN \quad (16)$$

$$\frac{dP}{dt} = Pup \times Pfb - PgP \quad (17)$$

where N_{up} and P_{up} are the nitrogen and phosphorus uptake rates, N_{fb} and P_{fb} are the nitrogen and phosphorus feed-back control.

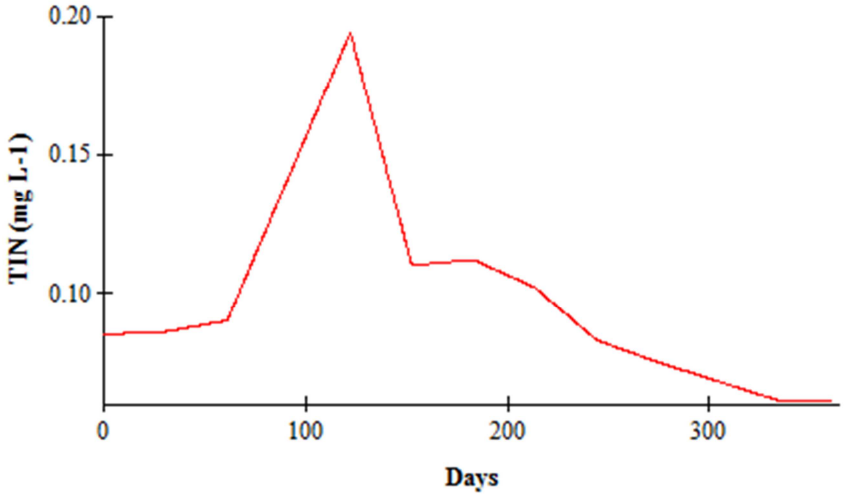

Figure 4.4 – Annual variation of total inorganic nitrogen (TIN) concentrations in Ria Formosa.

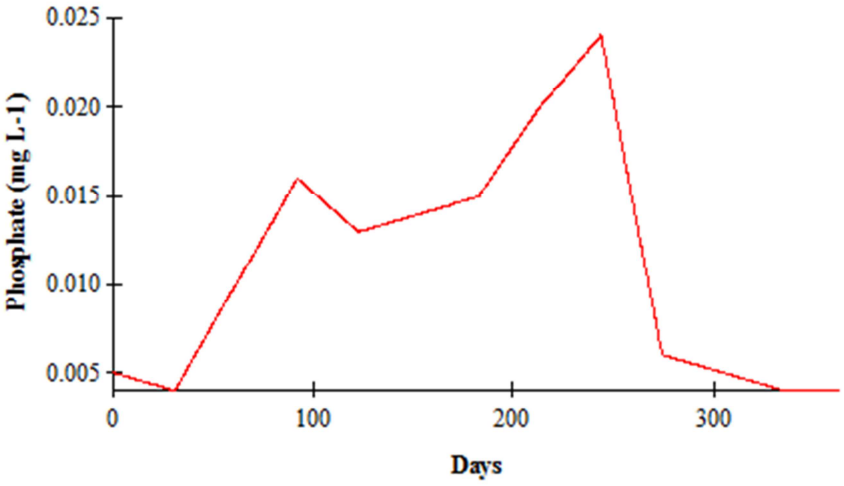

Figure 4.5 – Annual variation of phosphate concentrations in Ria Formosa.

2.3.1.1.2.1 Nutrient uptake

The equation used for nutrient uptake is a classic Michaelis-Menten (Van Den Hoek *et al.*, 1995):

$$N_{up} = VN_{max} \times \frac{TIN}{KN + TIN} \qquad (19)$$

$$P_{up} = VP_{max} \times \frac{HPO_4^{2-}}{Kp + HPO_4^{2-}} \quad (20)$$

where VN_{max} is the maximum uptake rate for nitrogen and VP_{max} is the maximum uptake rate for phosphorus, *TIN* is the total inorganic nitrogen in the water, HPO_4^{2-} is the phosphate concentration in the water, K_N and K_p are the half saturation constants for nitrogen and phosphorus uptake, respectively.

2.3.1.1.2.2 Nutrient feedback control

The equations used for nitrogen and phosphorus feedback control were obtained from Solidoro *et al.* (1997b), being set to:

$$N_{fb} = \frac{QN\max - N}{QN\max - QN\min} \quad (21)$$

$$P_{fb} = \frac{QP\max - P}{QP\max - QP\min} \quad (22)$$

where N is the internal quota of nitrogen, P is the internal quota of phosphorus, QN_{max}, QN_{min}, QP_{max} and QP_{min} are the maximum and minimum internal quota for nitrogen and phosphorus, respectively.

2.3.1.1.2.3 Nutrient limitation

The productivity limitation as a function of the internal quota of nitrogen *f(N)* and phosphorus *f(P)* at each time step was determined by the following expression:

$$f(N, P) = \min\left(\frac{N - QN\min}{QN\max - QN\min}, \frac{P - QP\min}{QP\max - QP\min}\right) \quad (23)$$

where N is the internal quota of nitrogen, P is the internal quota of phosphorus, *QN max*, *QN min*, *QP max* and *QP min* are the maximum and minimum internal quota for nitrogen and phosphorus, respectively.

2.3.2 Respiration

Respiration losses were considered to be associated only with the production processes. The respiratory rates were obtained from the laboratory incubation experiments and were considered to be constant over time.

2.3.3 Mortality

Natural mortality, *M*, was calculated according to a Michaelis-Menten type equation:

$$M = Max_{mort} \times (B/(P_g \times k_m + B)) \quad (24)$$

where Max_{mort} (d^{-1}) is the maximum mortality rate and K_m (d^{-1}) is the mortality rate.

2.4 Simulations

Seaweed productivity was simulated over a period of one year at the scale of 1 m^2 using different depths for each seaweed genus. The model was run with an hourly time step.

2.5 Model implementation

The present model was implemented in *Powersim*® 2.5, a visual simulation platform for construction of simple dynamic models.

2.6 Macroalgal Productivity in the Ria Formosa

To estimate the annual seaweed productivity in the Ria Formosa lagoon it was assumed that macroalgae occupy an area of 2.5 km^2, with 80% coverage of *Enteromorpha* spp. and 20% coverage of *Ulva* spp. (Aníbal, 1998), which corresponds to an area of 2 km^2 for *Enteromorpha* and of 0.5 km^2 for *Ulva* species.

3. Results and Discussion

To simulate macroalgal productivity, a suitable set of values for the parameters in the model had to be set up. Model parameters for *Enteromorpha* spp. and *Ulva* spp. are shown in Tables 3.1 and 3.2, respectively. Some parameters concerning seaweed productivity, like maximum production rates

and optimal light intensities were obtained from laboratory incubation experiments. Respiratory rates were obtained from *in situ* incubation experiments. Parameters related to nutrients, like the maximum and minimum internal concentrations for each macroalgae species, were obtained from an extensive literature search carried out in order to collect the information needed to give a range of suitable values for each parameter. Conversely to *Ulva* spp., a well-studied seaweed genus, data on *Enteromorpha* spp. was scarce (Fujita, 1985; Fong *et al*., 1994; Van Den Hoek *et al*. 1995). In some cases, *Enteromorpha* parameters (VP_{max} and K_p) were estimated from data on *Ulva*, because they are both opportunistic macroalgae, with similar behaviour in nutrients uptake. *Enteromorpha* mortality rate was also estimated from *Ulva* parameters, but a lower value was chosen, since the first has a higher capacity of resistance (Pregnall and Rudy, 1985).

3.1 Internal nutrient concentrations

The annual variation of internal nitrogen (*N*) and phosphorus (*P*) concentrations for *Enteromorpha* spp. are presented in Figs. 3.7 and 3.8, respectively. Internal *N* concentrations present great variability over the year, however the lowest values (below 40 mg N g^{-1} dw) occur during summer, conversely to external N concentrations, which were lower during winter (Fig. 3.4). Over the year, internal *N* concentrations are always higher than the minimum internal quota (10 mg N g^{-1} dw), suggesting that this nutrient is not limiting for the *Enteromorpha* primary productivity. Internal *P* concentrations follow the same pattern of annual variation as the external concentrations of this nutrient (Fig. 3.5), with highest values (1.3 mg P g^{-1} dw) during the spring/summer period and lowest values (below 0.5 mg P g^{-1} dw) during the winter period. This may be explained by the fact that during spring/summer, the algae store up phosphorus to be used during winter when the environmental conditions are ideal for photosynthesis.

Ulva internal concentrations of *N* and *P* are shown in Figures 3.9 and 3.10, respectively. The pattern of annual variation for *Ulva* internal *N* and *P* concentrations is similar to that observed for *Enteromorpha*, however the range of values for *Ulva* is lower. *Ulva* internal *N* ranges annually from 25 to 65 mg N g^{-1} dw, while for *Enteromorpha* the annual range of internal *N* concentrations is between 30 and 85 mg N g^{-1} dw. The maximum value of internal *P* for *Ulva* is 1.2 mg P g^{-1} dw, the same as for *Enteromorpha*, however the minimum value of internal *P* concentrations is lower for *Ulva* (0.2 mg P g^{-1} dw) than for *Enteromorpha* (0.4 mg P g^{-1} dw). Since the values of minimum nutrient internal quotas (Table 3.1 and 3.2) are similar for these seaweeds, the higher capacity of *Enteromorpha* to store up nutrients give them a competitive advantage over *Ulva* species, especially when external nutrient concentrations are limiting, thus explaining the higher biomasses documented for this macroalgae in the Ria Formosa (Aníbal, 1998).

Table 4.1 – Model parameters for *Enteromorpha* spp.

Symbol	Description	Unit	Literature range	Value used	Sources
P_{max}	Maximum production rate	mg C g^{-1} dw h^{-1}		3.55	Laboratory experiments
I_{opt}	Optimal light intensity	µE m^{-2} s^{-1}		97	Laboratory experiments
h	Average height of *Enteromorpha* stand above datum	m		1.2	Field data
N	Nitrogen internal concentration	mg N g^{-1} dw	10 - 30	20	Fong *et al.* (1994)
P	Phosphorus internal concentration	mg P g^{-1} dw	0 - 10	1	Fong *et al.* (1994)
VN_{max}	Maximum uptake rate for total inorganic nitrogen	mg N g^{-1} dw h^{-1}	1.68 – 13.9	1.68	Fujita (1985) Van Den Hoek *et al.* (1995)
VP_{max}	Maximum uptake rate for phosphates	mg P g^{-1} dw h^{-1}		0.23	
K_N	Half saturation constant for N	mg L^{-1}	0.2 – 0.6	0.25	Fujita (1985) Van Den Hoek *et al.* (1995)
K_P	Half saturation constant for P	mg L^{-1}		0.025	
QNmax	Maximum internal quota for nitrogen	mg N g dw^{-1}		35	Fong *et al.* (1994)
QNmin	Minimum internal quota for nitrogen	mg N g dw^{-1}		10	Fong *et al.* (1994)
QPmax	Maximum internal quota for phosphorus	mg P g dw^{-1}		4	Fong *et al.* (1994)
QPmin	Minimum internal quota for phosphorus	mg P g dw^{-1}		0.5	Fong *et al.* (1994)
R	Respiratory rate	mg C g^{-1} dw h^{-1}		0.04	*In situ* experiments
Max_{mort}	Maximum mortality rate	d^{-1}		0.2	
Km	Mortality rate	h^{-1}		0.003	

Table 4.2 – Model parameters for *Ulva* spp.

Symbol	Description	Unit	Literature range	Value used	Sources
P_{max}	Maximum production rate	mg C g^{-1} dw h^{-1}		3.66	Laboratory experiments
I_{opt}	Optimal light intensity	µE m^{-2} s^{-1}		72	Laboratory experiments
h	Average height of *Ulva* stand above datum	m		0.8	Field data
N	Nitrogen internal concentration	mg N g^{-1} dw		10	calibration
P	Phosphorus internal concentration	mg P g^{-1} dw		0.05	calibration
VN_{max}	Maximum uptake rate for total inorganic nitrogen	mg N g^{-1} dw h^{-1}	0.56 – 5.2	1	Lapointe and Tenore (1981); Lavery and McComb (1991); Fujita (1985)
VP_{max}	Maximum uptake rate for phosphates	mg P g^{-1} dw h^{-1}	0.23 – 1.09	0.23	Lavery and McComb (1991)
K_N	Half saturation constant for N	mg L^{-1}	0.2 - 0.6	0.25	Lavery and McComb (1991); Fujita (1985)
K_P	Half saturation constant for P	mg L^{-1}	0.025 – 0.113	0.025	Lavery and McComb (1991)
QNmax	Maximum internal quota for nitrogen	mg N g dw^{-1}	36 - 54	40	Rosemberg and Ramus (1982); Fujita (1985)
QNmin	Minimum internal quota for nitrogen	mg N g dw^{-1}	9 - 10	10	Rosemberg and Ramus (1982); Fujita (1985)
QPmax	Maximum internal quota for phosphorus	mg P g dw^{-1}		3.9	Coffaro and Sfriso (1997)
QPmin	Minimum internal quota for phosphorus	mg P g dw^{-1}	1.1	0.6	Coffaro and Sfriso (1997)
R	Respiratory rate	mg C g^{-1} dw h^{-1}		0.25	*In situ* experiments

Table 4.2 – (continuation).

Symbol	Description	Unit	Literature range	Value used	Sources
Max_{mort}	Maximum mortality rate	d^{-1}		0.08	
Km	Mortality rate	h^{-1}		0.005	Solidoro *et al.* (1997a)

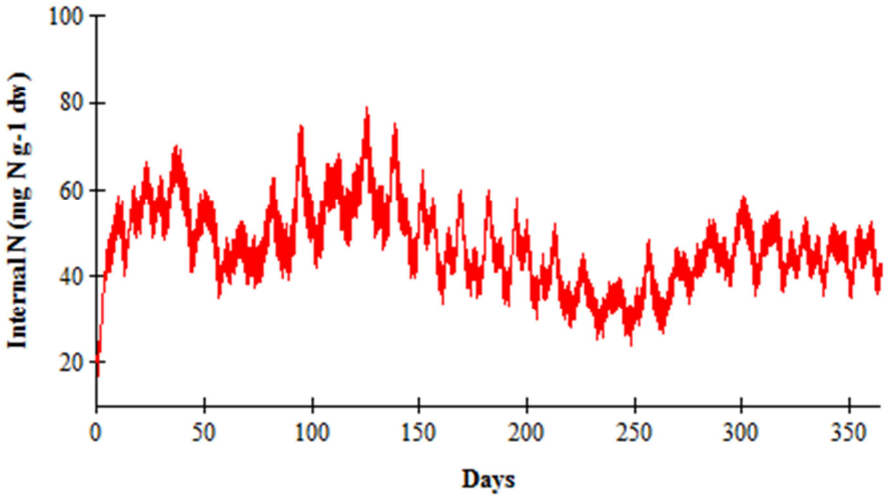

Figure 4.6 – Annual variation of internal nitrogen (N) concentrations for *Enteromorpha* spp.

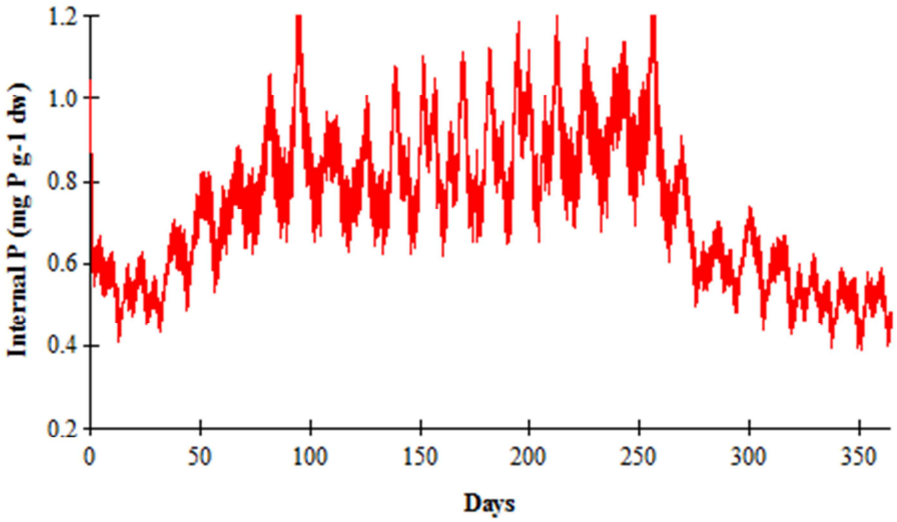

Figure 4.7 – Annual variation of internal phosphorus (P) concentrations for *Enteromorpha* spp.

As observed for *Enteromorpha*, internal *N* concentrations for *Ulva* over the year are always above the minimum internal quota (10 mg N g^{-1} dw) meaning that this nutrient is never limiting. These results may be due to the absence of inorganic nitrogen limitation in lagoon water, except for the areas close to seawater inlets (Newton *et al*., 2003), where there are practically no Ulvales due to the high hydrodynamic conditions. On the other hand, during winter, internal *P* concentrations are slightly below the minimum internal quota for both *Enteromorpha* (0.5 mg P g^{-1} dw) and *Ulva* (0.6 mg P g^{-1} dw), suggesting that seaweed productivity would be practically unaffected by *P* cell concentrations (Lavery and McComb, 1991). The lack of internal nutrient (*N* and *P*) limitation combined with ideal light intensities (below photoinhibition values) is probably the main cause for high seaweed photosynthetic rates during the autumn/winter period. During this season, the internal reserves of this nutrient are depleted due to external phosphorus limitation (Newton *et al*., 2003).

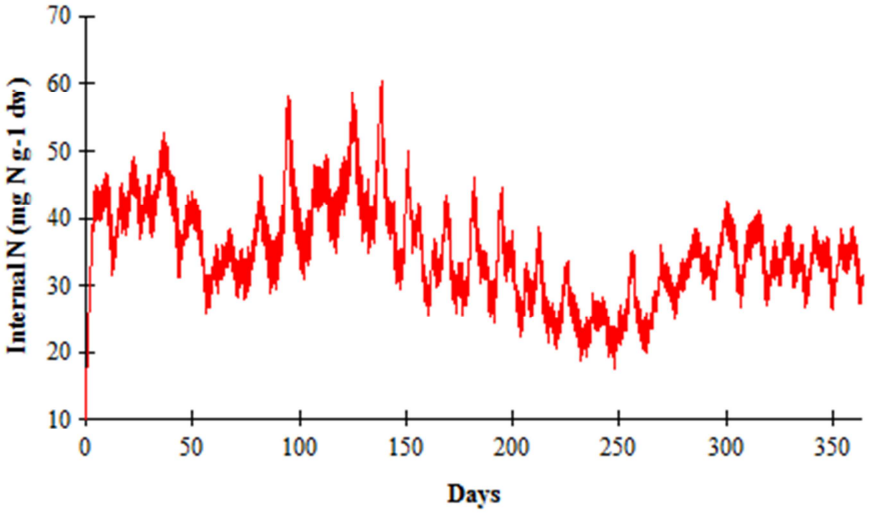

Figure 4.8 – Annual variation of internal nitrogen (N) concentrations for *Ulva* spp.

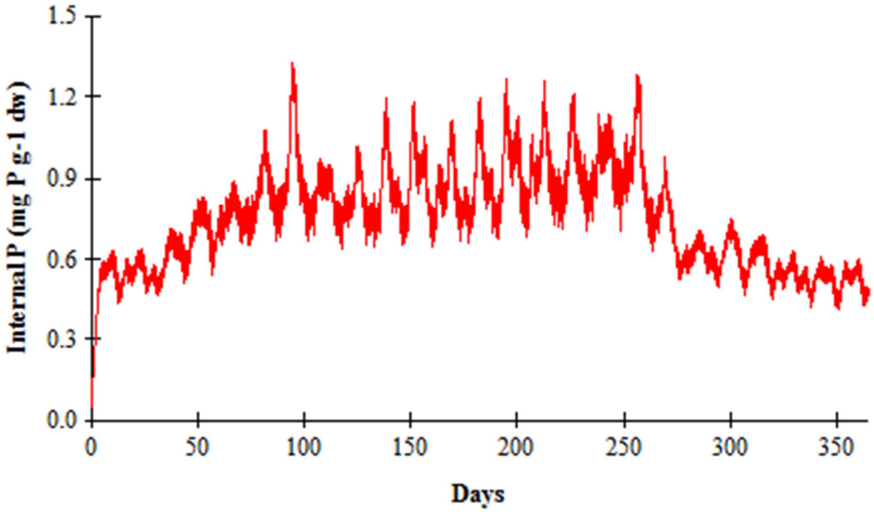

Figure 4.9 – Annual variation of internal phosphorus (P) concentrations for *Ulva* spp.

3.2 Primary Productivity

The results of annual gross primary production (P_g) and net primary production (P_n) per unit area obtained with the present model are presented in Table 3.3.

Table 4.3 – Annual gross primary production (P_g), net primary production (P_n) and turnover rates for each seaweed species. Data on biomass was obtained from Aníbal (1998) based on monthly measurements over a year.

Seaweeds	***Enteromorpha* spp.**	***Ulva* spp.**
Mean biomass (g C m^{-2})	22.4	5.7
P_g (gC m^{-2} y^{-1})	190	132
P_n (gC m^{-2} y^{-1})	162	98
Turnover P/B (y^{-1})	8	23

The model results show that *Enteromorpha* spp. has higher annual productivities than *Ulva* spp., as it would be expected because *Enteromorpha* lives in the upper zones of tidal flats, where light attenuation by the water column is reduced and consequently the light available for photosynthesis is higher. For *Ulva* species that live in deepest zones the PAR at the bottom of the water column is lower. The production values obtained by the model are lower than the ones referred by other authors (Ferreira, 1989; Alvera-Azcárate *et al*., 2003). For example, the Alvera-Azcárate *et al*. (2003) seaweed model estimated that the annual P_g for *Ulva lactuca* in the Tagus estuary was 609 g C m^{-2} y^{-1}, while annual P_n was 305 g C m^{-2} y^{-1}. For the same species and the same estuary, a model developed by Ferreira and Ramos (1989) yielded an annual P_g of 294 g C m^{-2} y^{-1} and an annual P_n of 213 g C m^{-2} y^{-1}. The higher values obtained with the referred models, may be related to the fact that photoinhibition was not taken in consideration; instead a light saturation function was used.

In fact, if instead of Steel's (1962) equation, a Michaelis-Menten type equation (saturation function) was used for light limitation, the annual P_g and P_n for *Enteromorpha* and *Ulva* estimated by the present model would be higher than the present values (Table 3. 4) and similar to those referred by Ferreira and Ramos (1989).

Turnover rates for *Enteromorpha* and *Ulva* are high, 8 y^{-1} and 23 y^{-1}, respectively, as it would be expected from opportunistic macroalgae, with fast growth. These values are comparable to those reported for green algae in the Tagus estuary (Alvera-Azcárate *et al*., 2003). *Ulva* species, with lowest biomasses, have higher turnover rates due to their higher productivity and capacity to colonize new substrates. In the Ria Formosa, *Ulva* species occupy lower intertidal areas where tidal

currents are stronger; thus they are constantly taken off and drifted away by the currents going to colonize other areas.

Table 4.4 – Annual gross primary production (P_g) and net primary production (P_n) calculated by the model, when a Michaelis-Menten type equation is used for light limitation.

Seaweeds	***Enteromorpha* spp.**	***Ulva* spp.**
Mean biomass (g C m^{-2})	22.4	5.7
P_g (g C m^{-2} y^{-1})	287	192
P_n (g C m^{-2} y^{-1})	252	150

3.3 Carbon and nutrient budgets

In order to estimate the annual contribution of seaweeds to the carbon fixation and nutrient removal in the Ria Formosa lagoon, the results of the small-scale productivity model were extrapolated in order to the determine the productivity for the whole system (Fig. 3.11).

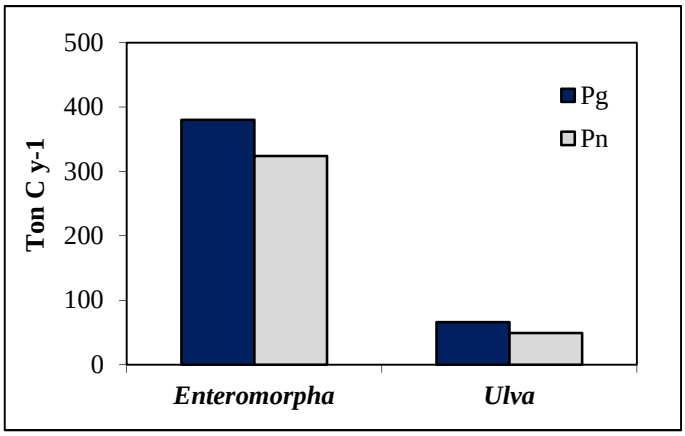

Figure 4.10 – Total annual seaweed production (Ton C y^{-1}) for the Ria Formosa lagoon.

The seaweed P_g in the Ria Formosa lagoon is 446 ton C y^{-1} and the P_n is 423 C y^{-1}. *Enteromorpha* species are the algae with the highest contribution (85%) to the system's carbon budget.

When the seaweed P_g results are applied to nutrient removal from lagoon water, it is possible to estimate the yearly N and P removal by these organisms (Table 3.5), considering the C:N:P ratios used by Alvera-Azcárate *et al*. (2003) for *Ulva lactuca*. The C:N:P ratios found in the present study

are lower than those reported by Atkinson and Smith (1983). According to the former authors, the C:N:P ratio for *Enteromorpha flexulosa* is 365:16:1, while for *Ulva* sp. it is 336:35:1.

Table 4.5 – Total yearly nitrogen and phosphorus removal (Ton y^{-1}) by *Enteromorpha* and *Ulva* species in the Ria Formosa lagoon.

Seaweeds	**C:N:P ratio**	**Total C production** (Ton y^{-1})	**Total N removal** (Ton y^{-1})	**Total P removal** (Ton y^{-1})
Enteromorpha spp.	45: 7:1	380	59	8.4
Ulva spp.	45:7:1	66	10	1.5
TOTAL		**446**	**69**	**9.9**

Annually, seaweeds remove 69 tons of N and 10 tons of P from the Ria Formosa lagoon. These quantities correspond in terms of population equivalents to 15700 inhabitants for N and 10000 inhabitants for P.

CHAPTER V.

General Conclusions

General Conclusions

The parameters of *P-I* curves obtained in the laboratory incubation experiments are useful for the parameterisation of macroalgal ecological models.

The model developed in the present work takes into account photoinhibition, which was observed in both *in situ* and laboratory productivity experiments. It also considers internal nutrient limitation because the highest *in situ* photosynthetic rates were observed during the winter period when there is phosphorus limitation in the lagoon water.

It is evident that the lack of internal nutrient (*N* and *P*) limitation combined with ideal light intensities (below photoinhibition values) is the main cause for high seaweed photosynthetic rates during the autumn/winter period. The capacity of opportunistic macroalgae to store up nutrients, when they are in excess, gives these algae a competitive advantage over other primary producers such as phytoplankton.

The model results could be further improved by using a complete year data sets for actual irradiances and external nutrient concentrations, in order to allow model validation. To improve the model laboratory experiments are also needed to study nutrient uptake kinetics and internal nutrient concentrations for the different macroalgae species. Further work may also include the upscaling of the model results to whole lagoon, using a Geographical Information System (GIS) and the coupling of this ecological model to a hydrodynamic model. Moreover, a sensitivity analysis should be performed in order to compare the results under different conditions and parameterisations.

Seaweeds have an important role in nutrient removal, acting as biofilters, which contribute to water quality improvement in coastal lagoons. They have also an important contribution to the ecosystem food web, as they have an important role in carbon fixation.

CHAPTER VI.

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